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A STUDY OF CERTAIN EFFECTS OCCASIONED IN DOGS BY DIPHTHERIA TOXIN

II. ANALYSIS OF THE MECHANISM POSSIBLY RESPONSIBLE FOR THE ALTERATIONS OF THE HEART *

HAROLD J. STEWART, M.D.

NEW YORK

In a preceding paper [1] were reported certain results that followed the injection of diphtheria toxin into dogs. Among the results reported were changes in the size of the heart. An analysis of these changes in size forms the subject of this paper.

Following the intravenous injection into dogs of 0.00135 cc. or more of diphtheria toxin per kilogram of body weight, the animals became ill, lost weight, presented jaundice, showed urinary changes indicating irritation of the kidneys and died from two to nineteen days after the injection. There was, in most of these dogs, progressive decrease in the amplitude of the R_2 and R_3 waves of the electrocardiogram. The ratio of the weight of the left ventricle of the heart to that of the right ventricle (called hereafter the L/R ratio) was below the average for the hearts of normal dogs in all except one animal (no. 109). The ratio of the combined left and right ventricular weights to the body weight (called hereafter the $\frac{L + R}{B W}$ ratio) in twelve dogs was below the normal average. In the remaining eight dogs, it was equal to the normal average.

The method of injecting the diphtheria toxin into the dog was described in the first paper.[1] Briefly, diphtheria toxin having a minimal lethal dose for guinea-pigs of 0.00125 cc. was injected intravenously through the marginal ear veins. The toxin was diluted with sterile physiologic sodium chloride solution, and the dose was calculated in cubic centimeters per kilogram of body weight. Electrocardiograms and roentgenograms of the heart were made, and the body weight was taken immediately before the injection of the toxin. These observations were repeated

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* From the Hospital of the Rockefeller Institute for Medical Research.

1. Stewart, H. J.: A Study of Certain Effects Occasioned in Dogs by Diphtheria Toxin: I. A Report of the Visceral Lesions, Arch. Path., to be published.

2. Levy, R. L.: The Size of the Heart in Pneumonia: A Teleroentgenographic Study, with Observations on the Effect of Digitalis Therapy, Arch. Int. Med. **32**:359 (Sept.) 1923.

3. Stewart, H. J.: A Technique for Measuring X-Ray Photographs of the Cardiac Areas of Dogs, J. Clin. Investigation **3**:475, 1927.

daily thereafter until the animals succumbed to the intoxication, or until further changes in those animals that survived were not observable. The roentgenograms of the heart were made at a distance of 2 meters, and the cardiac area was measured by the technic devised by Levy [2] and modified by Stewart [3] for use in dogs. The dogs were divided into two groups according to the amount of toxin injected per kilogram of body weight.

Four dogs (nos. 81, 82, 83 and 84 of group 1, table 1) were given from 0.00161 to 0.00232 cc. of diphtheria toxin per kilogram of body weight intravenously, and from one to four days later a second injection of from 0.00105 to 0.00168 cc. per kilogram of body weight. These

TABLE 1.—*The Effect of Diphtheria Toxin on the Cardiac Area and the Body Weight in Dogs of Group 1*

Dog	Time of Observation	Amount of Toxin Injected per Kg., Cc.	Died	Weight		Area of Heart		Per Cent Change in	
				Kg.	Per Cent of First Weight	Sq. Cm.	Per Cent of First Area	Weight	Area of Heart
81	Before injection	.....		10.75	100.0	41.40	100.0		
	After injection								
	1 day.....	0.00232 } 0.00337		9.80	91.2	29.63	71.3		
	2 days.....	0.00105 }	3d day	9.55	88.8	29.35	70.9	11.2	29.1
82	Before injection	.....		12.45	100.0	53.03	100.0		
	After injection								
	1 day.....	0.00161 }		11.75	94.4				
	2 days.....	0.00327		11.55	92.7	39.20	73.9		
	3 days.....			11.50	92.3	37.57	70.8		
	4 days.....	0.00166 }		10.85	87.1	37.50	70.7		
	5 days.....	.....	5th day	10.20	81.9	30.63	57.7	18.1	42.3
83	Before injection	.....		7.95	100.0	42.70	100.0		
	After injection								
	1 day.....	0.00166 } 0.00332		8.00	100.6	34.50	80.7		
	2 days.....	0.00166 }		7.80	98.1	34.20	80.0		
	3 days.....	.....	3d day	7.55	94.9	34.05	79.7	5.1	20.3
84	Before injection	.....		8.90	100.0	38.40	100.0		
	After injection								
	1 day.....	0.00168 } 0.00336		8.67	97.4	27.73	72.2		
	2 days.....	0.00168 }		8.37	94.1	27.55	71.7		
	3 days.....	.....	3d day	7.85	88.2	22.75	59.2	11.8	40.8

dogs exhibited a remarkable decrease in the size of the heart shadow, ranging from 20 to 42 per cent (fig. 1). The decrease was seen as early as twenty-four hours after the first injection. In dogs 81 and 83, it was at its maximum then, while in dogs 82 and 84 the area of the heart continued to decrease until the death of the animals, none of the animals surviving longer than five days after the first injection. This decrease in size is well seen in the roentgenograms of dog 82 (fig. 2).

The question arose: To what mechanism was this decrease in size of the heart due? A number of possibilities presented themselves. The decrease might have been due (1) to the decrease in body weight that also took place or (2) to the toxin injected. If due to the latter, there should be a dose that would fail to cause this change. The toxin could

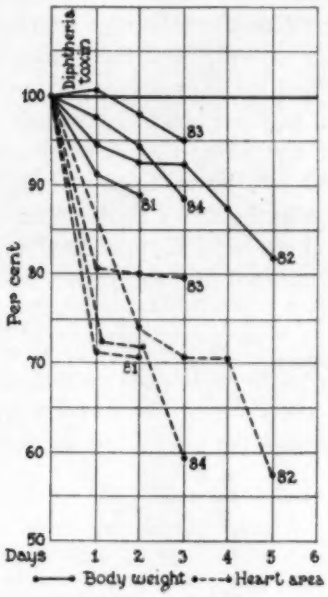

Fig. 1.—Graph showing the effect of diphtheria toxin on the cardiac area and the body weight in the dogs of group 1 (table 1). In this and in the succeeding figures, the lapse of days after the injection of the toxin is plotted on the abscissae. The numbers at the ends of the curves refer to the dogs.

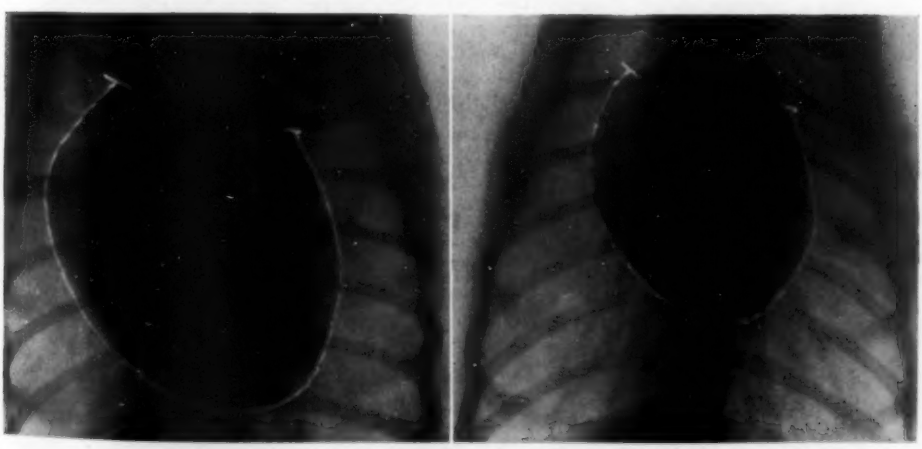

Fig. 2.—Roentgenogram of the heart of dog 82. *A* was taken Jan. 4, 1923, before, and *B* was taken Jan. 10, 1923, after a total of 0.00327 cc. of diphtheria toxin per kilogram of body weight had been given.

bring about the decrease in several ways: (1) by decreasing the total amount of the circulating blood, evidence of which might be found in a study of the number of red cells and the hemoglobin content of the blood, the blood volume and the microscopic sections of the organs after death; (2) by producing capillary dilatation, which would allow a redistribution of the blood in the body; (3) by destruction of the substance of the heart muscle, and (4) by an injury of the fibers of the heart muscle allowing a change in the water equilibrium, so that water would pass out of the cells and the cells be decreased in size. Each one of these possibilities was examined and the results are now reported.

OBSERVATIONS

Effect of a Decrease in Body Weight on the Area of the Heart.—In the dogs of group 1 there was a loss in body weight of from 11 to 18 per cent, and a decrease in the size of the heart of from 20 to 42 per

TABLE 2.—*The Effect of Fasting for Four Days on the Body Weight and the Cardiac Area in Normal Dogs*

Dog	Per Cent Change in Body Weight after Fasting Four Days	Per Cent Change in Area of Heart after Fasting Four Days
85 ♂	−12.0*	−7.6*
86 ♀	− 7.7	+5.5
87 ♀	− 6.5	−3.6
103 ♂	−10.3	−2.8
104 ♂	− 9.0	+8.1
105 ♂	−14.4	+6.9
106 ♀	−10.7	−3.6

* The negative sign indicates a decrease and the positive sign an increase.

cent (fig. 1). That the decrease in the area of the heart did not parallel and was not dependent on the loss in body weight is shown by the following experiments:

Seven dogs (nos. 85, 86, 87, 103, 104, 105 and 106) were not given food for from three to four days. They had water as desired. Roentgenograms were made before the period of fasting began and were repeated frequently throughout the period of fasting. The loss of body weight amounted to from 6 to 14 per cent (as recorded in table 2 and fig. 3 A), while the cardiac area varied between a decrease of 7 per cent and an increase of 8 per cent (table 2, fig. 3 B). These figures are within the limits of a variation of 10 per cent, which represents the error involved in the method. Although the fasting dogs showed approximately as great a decrease in body weight as did the dogs suffering from diphtheria intoxication, there was not a parallel decrease in the size of the heart. The loss in the dogs suffering from diphtheria intoxication, in all probability, was therefore due to the toxin and was not an aspect of the accompanying loss of body weight. The fasting dogs were later fed until they regained their former weight. They were then given diphtheria toxin. Promptly they showed a decrease in the size of the heart (fig. 2). Further evidence exhibiting the absence of parallelism between the loss of body weight and the decrease of cardiac area was furnished by the dogs in group 2 C (nos. 88, 89, 90 and 101 of table 3 and fig. 4). They received

only 0.001 cc. per kilogram of body weight, which was a dose too small to produce death. They showed as great a loss of weight as the dogs given the larger dose, yet a decrease in cardiac area did not accompany this loss in weight. In addition, dog 108 (table 4, fig. 5) which received 0.00135 cc. of toxin per kilogram of body weight did not show changes in body weight, but did show a decrease in the size of the heart.

Effect of Diphtheria Toxin on the Area of the Heart.—Since the dogs of group 1 that received a total of from 0.00327 to 0.00337 cc. of diphtheria toxin per kilogram of body weight showed striking decreases in cardiac area, it was important to learn how much toxin was necessary to bring about this condition. To acquire data on this point, I gave

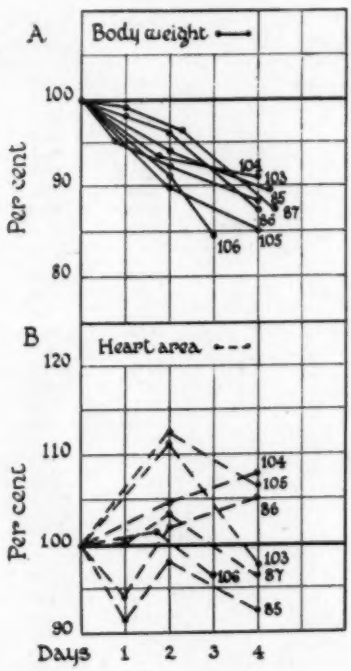

Fig. 3.—Graph *A* shows the effect of fasting on the body weight of dogs; graph *B*, the effect on the cardiac area.

decreasing dose of toxin to a second group of dogs, group 2 A being given 0.00168 cc., group 2 B, 0.00135 cc. and group 2 C, 0.001 cc. per kilogram of body weight.

In group 2 A, in which nine dogs (nos. 85, 86, 87, 97, 99, 102, 103, 104 and 105 of table 5) received, in a single injection, 0.00168 cc. per kilogram of diphtheria toxin, decrease in the area of the hearts promptly took place to the extent of from 19 to 39 per cent. The decrease in body weight was from 4 to 17 per cent (fig. 6). The animals died from two and a half to five days after the first injection. These changes were as great as those observed in the dogs receiving two injections (group 1).

TABLE 3.—*The Effect of Diphtheria Toxin on the Cardiac Area and the Body Weight in Dogs of Group 2 C*

Dog	Time of Observation	Amount of Toxin Injected per Kg., Cc.	Died	Weight		Cardiac Area	
				Kg.	Per Cent of First Weight	Sq. Cm.	Per Cent of First Area
88 ♂	Before injection	.....	.....	7.92	100.0	25.70	100.0
		0.001					
	After injection						
	1 day			7.46	94.1	27.35	106.4
	2 days			7.20	90.9	26.78	104.2
	3 days			7.20	90.9	27.75	107.9
	4 days			7.05	89.0	26.40	102.7
	6 days			7.20	90.9	28.18	109.7
	7 days			6.85	86.4	26.65	103.7
	9 days			6.80	85.8	27.95	108.7
	11 days			6.65	83.9	25.20	98.0
	14 days		Lived	6.80	90.9	27.30	106.2
89 ♀	Before injection	.....	.....	6.70	100.0	27.35	100.0
		0.001					
	After injection						
	1 day			6.43	95.9	26.18	95.7
	2 days			6.40	95.5	26.50	96.9
	3 days			6.50	97.0	26.40	96.5
	4 days			6.35	94.7	28.68	104.8
	6 days			6.15	91.7	27.80	101.6
	7 days			5.85	87.3	25.85	98.1
	9 days			5.90	88.0	25.35	92.6
	11 days			5.75	85.8	28.60	104.5
	14 days		Lived	6.10	91.0	25.65	93.7
90 ♂	Before injection	.....	.....	11.20	100.0	39.55	100.0
		0.001					
	After injection						
	1 day			11.15	99.5	37.30	94.5
	2 days			11.05	98.6	39.70	100.3
	3 days			10.95	97.7	41.30	104.4
	4 days			10.70	95.5	40.45	102.2
	6 days			10.20	91.0	37.60	95.0
	7 days			10.05	89.7	37.15	93.9
	9 days			9.70	86.6	36.75	92.9
	11 days			9.95	88.8	41.30	104.4
	14 days		Lived	10.20	91.0	38.35	96.9
101 ♀	Before injection	.....	.....	12.30	100.0	39.40	100.0
		0.001					
	After injection						
	1 day			12.00	97.5	39.80	101.0
	2 days			11.90	96.7	37.60	95.4
	3 days			12.25	99.6	43.45	109.7
	4 days			11.65	94.7	42.80	108.6
	5 days			11.90	96.7	45.55	118.1
	7 days			11.15	90.6	43.65	110.7
	10 days			10.43	84.7	38.20	96.9
	15 days		Lived	11.82	96.1	39.10	99.2
106 ♀	Before injection	.....	.....	11.57	100.0	39.35	100.0
		0.001					
	After injection						
	1 day			11.50	99.4	38.30	97.3
	2 days			11.30	97.6	36.75	93.4
	3 days			10.81	93.4	34.75	88.3
	4 days			11.11	96.0	32.40	82.3
	6 days			10.39	89.8	34.75	88.3
	7 days			9.98	86.2	35.40	89.9
	8 days			9.67	83.5	31.80	80.8
	9 days			9.40	81.2	33.40	84.8
	14 days			7.10	61.3	33.70	85.6
	20 days			7.50	64.8	41.75	106.0
	23 days		23d day	7.05	60.9	37.75	94.9

TABLE 4.—*The Effect of Diphtheria Toxin on the Cardiac Area and the Body Weight in the Dogs of Group 2 B*

Dog	Time of Observation	Amount of Toxin Injected per Kg., Cc.	Died	Weight		Cardiac Area		Per Cent Change in	
				Kg.	Per Cent of First Weight	Sq. Cm.	Per Cent of First Area	Weight	Cardiac Area
107 ♂	Before injection	.....	.....	17.18	100.0	66.50	100.0		
		0.00135							
	After injection								
	1 day			17.41	101.3	51.10	76.8		
	2 days			15.80	91.9	50.45	75.8		
	3 days			15.83	92.1	56.45	84.8		
	5 days			15.96	92.3	55.85	83.9		
	6 days			16.00	93.1	51.10	76.8		
	8 days			15.30	89.0	50.70	76.3		
	9 days			14.90	86.7	47.70	71.7		
	10 days			14.51	84.4	42.80	64.3		
	12 days			14.10	82.0	40.25	60.5		
	13 days			13.90	80.9	43.30	65.1		
	14 days			13.85	80.6	42.65	64.1	19.4	35.9
	15 days		16th day	13.50	78.5	*			
108 ♂	Before injection	.....	.....	19.15	100.0	57.80	100.0		
		0.00135							
	After injection								
	1 day			19.22	100.3	59.70	103.2		
	2 days			19.55	102.0	55.60	96.1		
	3 days			19.40	101.3	48.60	84.0	+1.3	16.0
	4 days		4th day						
109 ♂	Before injection	.....	.....	15.38	100.0	51.85	100.0		
		0.00135							
	After injection								
	1 day			15.25	99.1	49.90	96.2		
	2 days			15.35	99.8	43.80	84.4		
	3 days			14.93	97.0	45.55	87.8		
	5 days			14.24	92.5	41.80	80.6		
	6 days			14.65	95.2	47.60	91.8		
	8 days			14.30	92.9	49.45	95.3		
	9 days			13.85	90.1	51.60	99.5		
	10 days			13.50	87.7	47.70	91.9		
	12 days			13.27	86.3	45.55	87.8		
	13 days			12.90	83.8	43.00	82.9		
	14 days			12.85	83.5	38.50	74.2		
	15 days			12.55	81.6	46.70	90.2		
	16 days			12.40	80.6	42.20	81.3		
	17 days		18th day	12.03	78.2	43.20	83.3	21.8	16.7
110 ♂	Before injection	.....	.....	13.85	100.0	48.80	100.0		
		0.00135							
	After injection								
	1 day			14.15	102.1	41.35	84.7		
	2 days			13.88	100.2	42.25	86.5		
	3 days			13.45	97.1	45.30	92.8		
	5 days			12.90	93.1	43.65	89.3		
	6 days			12.85	92.7	41.25	84.5		
	8 days			12.10	87.4	42.40	86.8		
	9 days			11.80	85.1	43.70	89.5		
	10 days			11.40	82.3	32.90	67.4		
	11 days			10.97	79.9	33.15	67.9		
	13 days			10.85	78.3	31.00	63.5	21.7	36.5
	14 days			10.75	77.6	32.90	67.4		
	15 days			10.53	76.0	33.45	68.5		
	16 days			10.38	74.9	35.55	72.8		
	17 days			10.25	74.0	34.15	69.9	26.0	30.1
	19 days			9.96	71.8	*			
	20 days		20th day	9.65	69.6	*			
111 ♂	Before injection	.....	.....	16.45	100.0	49.70	100.0		
		0.00135							
	After injection								
	1 day			16.08	97.7	44.50	89.5		
	2 days			15.85	96.3	41.85	84.3		
	3 days			15.70	95.2	42.20	84.9		
	5 days			15.55	94.5	43.35	87.2		
	6 days			15.18	91.6	37.90	76.3	8.4	23.7
	8 days			14.80	89.9	41.80	84.1		
	9 days			14.05	85.4	40.50	81.0		
	10 days		11th day	13.64	82.9	41.90	84.3	17.1	15.7

* The heart shadow was not distinct enough to be outlined.

TABLE 4.—*The Effect of Diphtheria Toxin on the Cardiac Area and the Body Weight in the Dogs of Group 2 B—Continued*

Dog	Time of Observation	Amount of Toxin Injected per Kg., Cc.	Died	Weight		Cardiac Area		Per Cent Change in	
				Kg.	Per Cent of First Weight	Sq. Cm.	Per Cent of First Area	Weight	Cardiac Area
112 ♂	Before injection	.....	.. ...	14.70	100.0	57.95	100.0		
	After injection	0.00135							
	1 day			15.00	102.0	50.15	86.5		
	2 days			15.12	102.8	50.85	87.7		
	3 days			14.88	101.2	57.90	99.9		
	5 days			15.15	103.0	53.60	92.4		
	6 days			14.62	99.4	49.40	85.2		
	8 days			14.20	96.5	50.70	87.4		
	9 days			13.65	92.8	50.38	86.7		
	10 days			13.02	88.5	43.80	75.5		
	12 days		13th day	12.55	85.3	43.90	75.7	14.7	24.3

In group 2 B, in which six dogs (nos. 107, 108, 109, 110, 111 and 112 of table 4) received 0.00135 cc. per kilogram of toxin, dog 108 became acutely ill and died on the fourth day after the injection; the other five dogs gradually became ill and died from twelve to nineteen days after the injection. The decrease in cardiac area was, on the average, as great as in those receiving the larger doses. In four dogs (nos. 107, 110, 111 and 112 of fig. 5B) the decrease was shown on the day following the injection, and in two dogs (nos. 108 and 109) a definite decrease in cardiac size did not take place until the second or third days after the injection. The size of the heart did not decrease as steadily as in the dogs receiving 0.00168 cc. per kilogram of body weight; the decrease was more gradual and fluctuated (as in dog 109). The loss of body weight observed in these dogs was greater than that observed in the other groups, presumably because the animals survived for a longer time (fig. 5A).

In group 2C, in which five dogs (nos. 88, 89, 90, 101 and 106 of table 3) received 0.001 cc. of toxin per kilogram of body weight, the clinical course of dog 106, which survived the injection only twenty-three days, was somewhat similar to that of the animals in group 2B; the other four dogs did not show decreases in cardiac size (fig. 4B), although they lost as much weight immediately following the toxin injections as did the dogs given the larger dosages (fig. 4A). These dogs were living and well twelve months after receiving the toxin.

Summary: From these experiments, it is clear that the decrease in the size of the heart was dependent on the diphtheria toxin. Doses of 0.00135 cc. or more per kilogram of body weight resulted in decreases in the area of the heart, while doses of 0.001 cc. per kilogram of body weight were without effect.

Effect of Diphtheria Toxin on the Amount of the Circulating Blood.—I next studied the animals with a view to learning whether a decrease in the amount of the circulating blood took place. These studies were made in dogs into which had been injected 0.001 and 0.00168 cc. of toxin per kilogram of body weight.

Method: To ascertain whether blood destruction occurred after the injection of the diphtheria toxin, I counted the red blood cells and made estimations of the hemoglobin (expressed as oxygen capacities). As these do not give any indication of the volume of whole blood in circulation, estimations of blood volume were

also carried out. The red cells were counted in blood obtained from the ear by a needle. The blood used for the estimations of oxygen capacity was taken from that drawn from a femoral artery in the course of the measurements of the blood volume. The analyses for oxygen capacity were made according to a method described by van Slyke and Neill,[4] the van Slyke manometric apparatus being used. The estimations of the blood volume were made by the vital red method introduced by Keith, Rowntree and Geraghty.[5]

Vital red was used in 1.5 per cent solution in freshly distilled water. Of this solution, 1 cc. was injected for each 5 Kg. of body weight. The standard

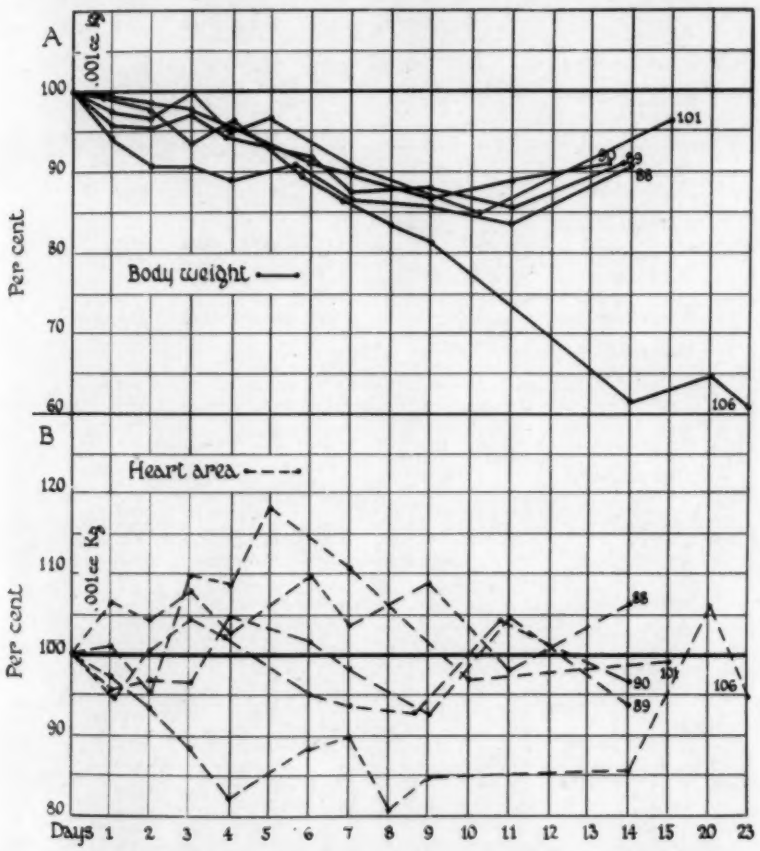

Fig. 4.—Graph *A* shows the effect of diphtheria toxin on the body weight and graph *B* the effect on the cardiac area in the dogs of group 2 C (table 3).

was made of one part of dye diluted 1 : 200, one part control plasma (that is, plasma of the blood taken before the injection of the dye) and two parts of 0.9 per cent sodium chloride. The blood used as control was drawn from a femoral

4. Van Slyke, D. D., and Neill, J. M.: The Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement, I. J. Biol. Chem. **61**:523, 1924.

5. Keith, N. M.; Rowntree, L. G., and Geraghty, J. T.: A Method for the Determination of Plasma and Blood Volume, Arch. Int. Med. **16**:547 (Oct.) 1915.

TABLE 5.—*The Effect of Diphtheria Toxin on the Cardiac Area and the Body Weight in the Dogs of Group 2 A*

Dog	Time of Observation	Amount of Toxin Injected per Kg., Cc.	Died	Weight		Cardiac Area		Per Cent Change in	
				Kg.	Per Cent of First Weight	Sq. Cm.	Per Cent of First Area	Weight	Cardiac Area
85 ♂	Before injection	.....	.....	7.00	100.0	27.70	100.0		
	After injection	0.00168*							
	1 day			6.30	90.0	28.50	102.8		
	2 days			6.30	90.0	24.25	87.5		
	3 days			6.05	86.4	23.63	85.3		
	5 days			5.80	82.8	19.55	70.5	17.2	29.5
	6 days			5.62	80.3	20.20	72.8		
	7 days			5.62	80.3	25.80	93.1		
	8 days		8th day	5.50	78.5	27.50	99.2	21.5	0.8
86 ♀	Before injection	.....	.....	9.77	100.0	35.40	100.0		
	After injection	0.00168							
	1 day			9.00	93.1	29.70	83.8		
	2 days			8.80	90.0	24.40	68.9	10.0	31.1
	3 days		3d day	8.55	87.5	25.65	72.4	12.5	27.6
87 ♀	Before injection	.....	.....	16.10	100.0	51.75	100.0		
	After injection	0.00168							
	1 day			15.15	94.0	45.40	87.7		
	2 days			14.45	89.7	33.50	64.7		
	3 days		3d day	13.90	86.3	33.35	64.4	13.7	35.6
97 ♀	Before injection	.....	.....	16.20	100.0	51 65	100.0		
	After injection	0.00168							
	1 day			16.00	98.7	43.15	83.5		
	2 days			15.60	96.3	38.50	74.5		
	3 days		3d day	15.50	95.7	34.70	67.2	4.3	32.8
99 ♂	Before injection	.....	.....	14.00	100.0	57.15	100.0		
	After injection	0.00168							
	1 day			13.40	95.7	50.48	88.3		
	2 days			13.25	94.6	43.80	76.6		
	3 days			13.30	95.0	39.75	69.5	5.0	30.5
	4 days			13.20	94.3	42.05	73.5		
	5 days		5th day	13.20	94.3	40.60	71.0	5.7	29.0
102 ♂	Before injection	.....	.....	14.00	100.0	52.35	100.0		
	After injection	0.00168							
	1 day			13.70	97.9	32.75	62.5		
	2 days			13.50	96.4	39.60	75.6		
	3 days			13.10	93.6	34.80	66.4		
	4 days		4th day	12.30	87.9	31.70	60.5	12.1	39.5
103 ♂	Before injection	.....	.....	11.20	100.0	38.45	100.0		
	After injection	0.00168							
	1 day			10.85	96.8	31.50	81.9		
	2 days			10.95	97.7	32.70	80.4		
	3 days			10.75	95.9	35.60	92.5		
	4 days		4th day	10.32	92.1	25.75	66.9	7.9	33.1
104 ♂	Before injection	.....	.....	9.02	100.0	36.45	100.0		
	After injection	0.00168							
	1 day			8.76	97.1	34.10	93.5		
	2 days		2d day	8.70	95.7	28.40	77.9	4.3	22.1
105 ♂	Before injection	.....	.....	11.12	100.0	43.85	100.0		
	After injection	0.00168							
	1 day			10.80	97.1	36.10	82.3		
	2 days			11.40	102.5	37.35	85.1		
	3 days			10.95	98.4	38.10	86.8		
	4 days			10.92	98.2	34.00	77.5	1.8	20.5
	5 days		5th day	10.60	95.3	35.70	81.4	4.7	18.6

* The dog moved during the injection, so that about one half was given subcutaneously.

artery; the dye was then injected into a superficial vein of that same leg; in from four to five minutes, the second sample of blood was drawn from the opposite femoral artery. The femoral artery was used for obtaining the sample because it is superficial in dogs; it is easily punctured with a needle and as large a sample may be obtained as is necessary. Stasis is thereby avoided. Pressure was applied for a few minutes over the artery after the removal of the needle to prevent extravasation of blood into the tissues. Potassium oxalate was used as an anticoagulant. Hematocrit readings were made after three Epstein tubes had been filled, one with each specimen of blood, and centrifuged until further centrifugation did not give any change in the packing of the cells.

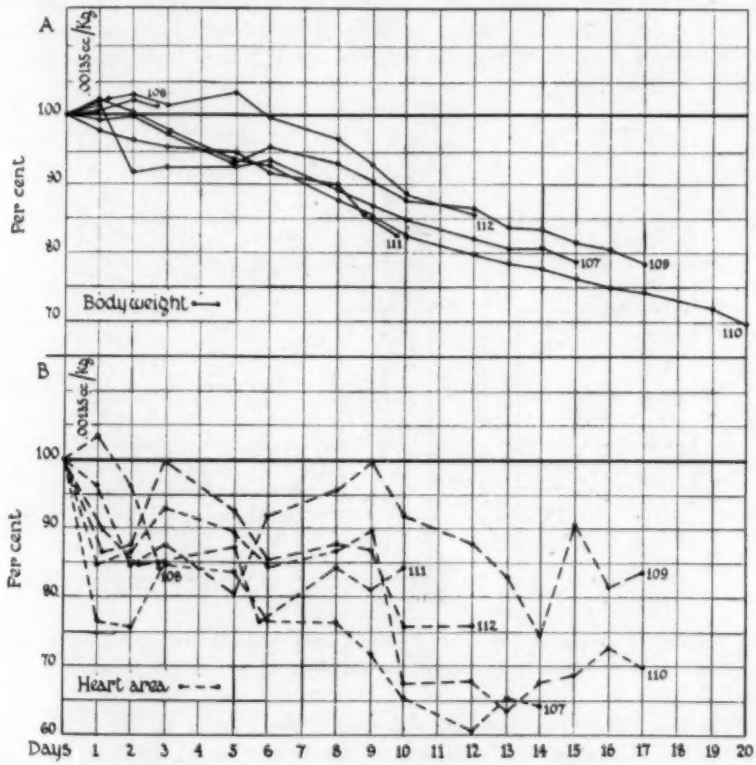

Fig. 5.—Graph *A* shows the effect of diphtheria toxin on the body weight, and graph *B* that on the cardiac area in the dogs of group 2 B (table 4).

Effect of Diphtheria Toxin on the Number of Red Cells and Hemoglobin: Five animals in group 2A that exhibited decrease in size of the heart did not show any change in the red blood cell count following the injection of 0.00168 cc. of diphtheria toxin per kilogram of body weight (dogs 97, 99, 102, 103 and 105 of table 6 and fig. 7). One of the dogs (no. 97) did not show any change in the oxygen capacity of the blood, the other four showed a slight increase. Of the two animals that received 0.001 cc. per kilogram of body weight, a dose that was without effect on the size of the heart, one (dog 101) showed a 33 per cent decrease in the red blood cell count with a smaller decrease of 6 per cent

in the oxygen capacity. The other animal (dog 106) did not show any change in the red blood cell count and the capacity for oxygen. Dogs 99, 102 and 103, which were given 0.00168 cc. toxin per kilogram of body weight exhibited an increase in the proportion of cells to plasma, according to the hematocrit readings; dogs 97 and 105, which received the same amount of toxin per kilogram of body weight, and dogs 101 and 106, which received 0.001 cc. per kilogram, did not show any deviation in the hematocrit readings. The hematocrit readings agree with the estimations of capacity for oxygen because they were made from arterial blood; in those instances in which there is a divergence of the red cell counts from the figures that one would expect from the oxygen capacity and the hematocrit readings, it is likely that the discrepancy is due to the fact that the blood for the red blood cell counts was taken from the superficial vessels of the ear, where the concentration of cells was possibly different from the concentration in the arterial blood.

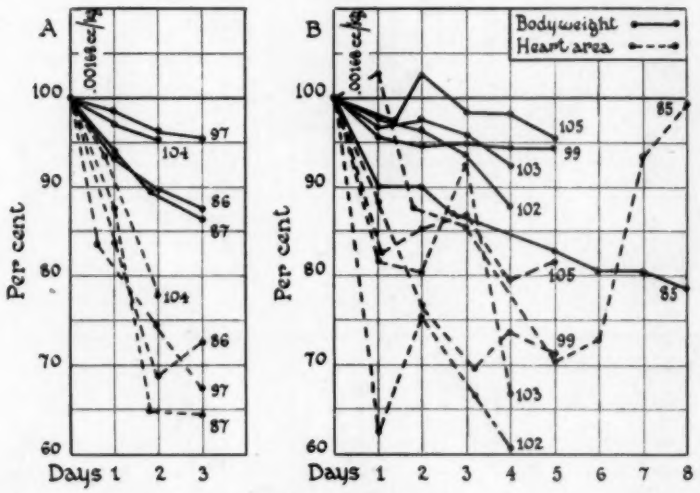

Fig. 6.—Graph *A* shows the effect of diphtheria toxin on the cardiac area, and graph *B* the effect on the body weight in the dogs of group 2A (table 5).

Summary: There was, then, no decrease in the number of red blood cells, in the amount of hemoglobin or in the relative proportion of cells to plasma in five dogs in which there was a decrease in cardiac area. One of two dogs that received a dose of toxin too small to affect the area of the heart, showed a slight decrease in the number of red blood cells and in the amount of hemoglobin, while the other dog did not show any change. These data indicate that destruction of red blood cells was not a factor in the mechanism responsible for the decrease in the cardiac area.

Effect of Diphtheria Toxin on the Blood Volume: Estimations of blood volume were made on five dogs before and after injections of diphtheria toxin. The total blood volume of dog 101 (table 6, fig. 7) was 1,282 cc. before the injection of toxin; three days after the injection of toxin, it was 1,041 cc. This dog, which had been given 0.001 cc. per kilogram of body weight and which showed a decrease of 18 per cent in blood volume, did not present any significant change in the size of the heart. Before the injection of toxin into dog 102, the blood

volume was 1,864 cc. It was 1,739 cc. three days after the injection, a decrease of 6 per cent (this is within the limits of error of the method) at a time when the cardiac area was decreased 33 per cent. The estimations for dog 103 revealed an increase in the total blood volume of 26 per cent on the third day after the injection of toxin, while the cardiac area showed a decrease of 19 per cent. There was an increase of 6 per cent in the blood volume of dog 105, while the area of the heart was decreased 13 per cent from that before the injection. Dog 106 showed a decrease of 12 per cent in the total blood volume accompanying a decrease of 11 per cent in the area of the heart.

TABLE 6.—*The Effect of Diphtheria Toxin on the Blood Volume, the Number of Red Blood Cells, the Hemoglobin and the Size of the Heart in Dogs*

Dog	Time of Observation	Hematocrit Reading, per Cent Cells	Red Cell Count, Millions	Hemoglobin of Oxygen Capacity per Cent by Volume	Plasma, Cc.	Whole Blood, Cc.	Cardiac Area, Sq. Cm.	Amount of Toxin Injected per Kg., Cc.
97	Before injection.....	41.6	8.9	21.52	.....	.....	51.65	
	Third day after.....	43.5	9.2	21.66	.....	.....	34.70	0.00168
	Percentage change*...	+1.9	+3.3	+0.6	.....	.....	−32.8	
99	Before injection.....	43.8	8.7	23.50	.....	.....	57.15	
	Third day after.....	55.5	8.5	28.62	.....	.....	39.75	0.00168
	Percentage change....	+11.7	−2.3	+21.2	.....	.....	−30.5	
102	Before injection.....	39.2	10.1	21.06	1,136	1,864	52.35	
	Third day after.....	50.9	9.7	22.96	854	1,739	34.80	0.00168
	Percentage change....	+11.7	−4.0	+9.4	−21.9	−6.7	−33.6	
103	Before injection.....	43.3	8.3	20.98	608	1,074	38.45	
	Second day after.....	51.5	8.3	24.40	658	1,357	32.70	0.00168
	Percentage change....	+8.2	0.0	+16.3	+8.2	+26.3	−19.6	
104	Before injection.....	41.4	7.7	21.72	657	1,121	35.45	
	Second day after.....	....	....	.....	.....	.....	28.40	0.00168
	Percentage change....	....	....	.....	.....	.....	−22.1	
105	Before injection.....	37.3	6.8	17.01	793	1,265	43.85	
	Third day after.....	39.0	7.0	18.40	823	1,349	38.10	0.00168
	Percentage change....	+1.7	+2.9	+8.1	+3.7	+6.6	−13.2	
106	Before injection.....	48.3	8.5	22.78	1,032	1,906	39.35	
	Third day after.....	49.7	8.7	22.16	871	1,731	34.75	0.001
	Percentage change....	+1.4	+2.3	−2.7	−15.6	−12.8	−11.7	
101	Before injection.....	24.9	7.1	10.18	964	1,282	39.40	
	Third day after.....	23.6	4.7	9.57	795	1,041	43.45	0.001
	Percentage change....	−1.3	−33.8	−6.0	−17.6	−18.8	+9.7	

* The positive sign indicates an increase; the negative sign a decrease.

Summary: Following the injection of diphtheria toxin, one dog showed a decrease of blood volume and not any change in the size of the heart; two did not show any change in blood volume and did show decreases in the cardiac area; one showed an increase in blood volume and a decrease in cardiac area, and one, a slight decrease in blood volume accompanying a slight decrease in cardiac area (table 7).

Of the four dogs showing a decrease in cardiac area, only one showed a decrease in total blood volume. From these few experiments, the limits of error of the method of estimating blood volume being taken into consideration, the volume does not appear to have changed and therefore could not have been a factor in bringing about the decrease in cardiac size.

Effect of a Change in the Blood Volume on the Area of the Heart: On account of the lack of relationship between the cardiac size and the blood volume

in these experiments, it seemed important to learn precisely what the effect on the size of the heart is when the blood volume is altered. The following experiment was devised to show the effect of a change in blood volume on the size of the heart. A known amount of blood was taken from a dog, and the effect on the size of the heart was observed. The blood withdrawn from this dog (no. 1) was transfused into another dog (no. 2), and the effect of the increase in blood volume was observed. Dog 116, weighing 13.44 Kg., presumably had a total blood volume of approximately 1,200 cc. After a roentgenogram of the heart had been taken, 500 cc. of blood was removed from the left femoral artery. The dog was in place on the roentgen-ray table during this procedure. Roentgenograms were made immediately after the blood had been withdrawn, at short intervals during that day, and then daily. Counts of the red blood cells were made at the same time that the roentgenograms were made. There was a sharp fall of 20 per cent (table 8; fig. 8A) in the cardiac area immediately after the

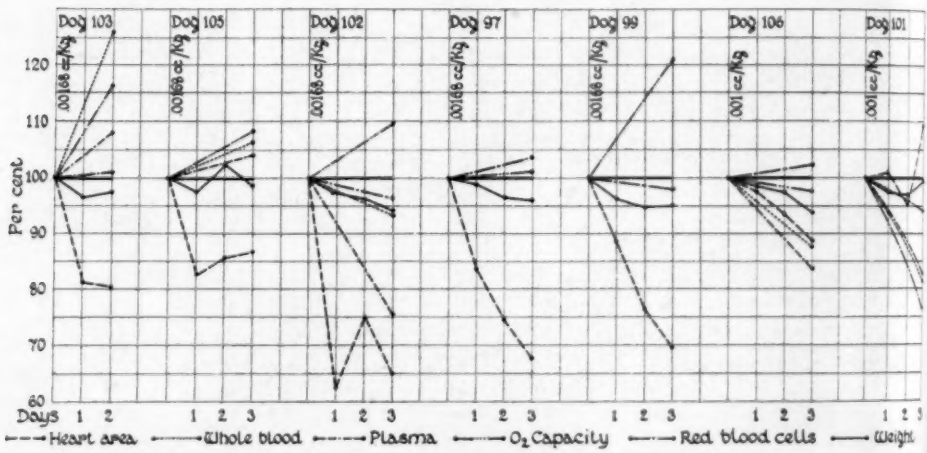

Fig. 7.—A graph showing the effect of diphtheria toxin on the hemoglobin, the number of red blood cells and blood volume in dogs (table 6). The cardiac areas and the body weights of the corresponding dogs are also plotted.

blood had been removed, and this fell further to 25 per cent in two hours and forty minutes. The count of the red blood cells did not show any change until the day after the bleeding, then the count was found to have decreased 17 per cent and the following day 30 per cent, probably owing to the dilution of the blood in the body's attempt to restore the blood volume to normal. The cardiac area showed a decrease of from 20 to 30 per cent for six days; it then returned to 88 per cent of the area first observed, and remained at this figure for one month. At the time of the return of the cardiac area to 88 per cent of its initial size, the count of the red blood cells had still further decreased, indicating a further dilution of the blood.

The 500 cc. of blood removed from dog 116 was collected under sterile procedure. Clotting was prevented by the addition of 3 per cent sodium citrate to a final dilution of 0.3 per cent. This blood was transfused into dog 115, weighing 11.96 Kg. The blood of dog 115 had beforehand been cross-agglutinated

against the blood of dog 116 by the rapid method of Rous and Turner.[6] Dog 115 presumably had a blood volume of approximately 1,000 cc.; by the addition of 500 cc. of blood, the total blood volume was increased about 50 per cent. Roentgenograms and counts of the red blood cells were made immediately after the transfusion and at short intervals later. Immediately after the transfusion was completed, the cardiac area increased 8 per cent (table 9; fig. 8B). The next day it was only 2 per cent greater than it had been in the beginning. Since the position of the dog was not changed between the taking of the first roentgenogram and that immediately after the transfusion, this 8 per cent increase in size is significant. Three days after the transfusion, the area had decreased to

TABLE 7.—*Summary of the Changes in Blood Volume with the Corresponding Changes in Cardiac Area in Dogs Following the Injection of Diphtheria Toxin*

Dog	Cardiac Area	Blood Volume
101	No change	Decrease
102	Decrease	No change
103	Decrease	Increase
105	Decrease	No change
106	Decrease	Decrease

TABLE 8.—*The Effect of Decreasing the Blood Volume by 500 Cc. on the Cardiac Area and the Number of Red Blood Cells (Dog 116)*

	Weight		Cardiac Area		Red Cells	
Time of Observation	Kg.	Per Cent of First Weight	Sq. Cm.	Per Cent of First Area	Millions	Per Cent of First Count
Before bleeding	13.44	100.0	47.80	100.0	7.2	100.0
Immediately after bleeding	.....	.....	38.60	80.7	...	.....
Hours after bleeding						
1.6	.....	.....	36.20	75.7	6.8	94.4
2.6	.....	.....	35.45	74.1	7.0	97.2
6.1	.....	.....	42.35	88.5	6.9	95.9
21	12.27	91.3	37.20	77.8	7.0	97.2
27	.....	.....	39.60	82.8	6.0	83.3
Days after						
2	12.02	89.4	37.45	78.3	5.1	70.8
3	12.40	93.0	34.00	71.1	5.7	79.1
5	12.75	94.8	39.10	81.5	5.6	77.7
6	12.15	90.4	36.70	76.6	5.5	76.3
7	12.75	94.8	39.60	82.8	...	....
8	12.25	91.1	42.20	88.2	4.2	58.3

91 per cent of its original size, and on the fifth day to 83 per cent. The cause of the secondary decrease is not clear. The count of the red blood cells increased 25 per cent immediately after the transfusion and, with slight fluctuations, continued to rise until the eighth day, when the increase reached 42 per cent. The amount of the urine of this dog was large during the first two days after the transfusion.

Summary: It may be seen, then, that a decrease in blood volume decreased the size of the heart, and a corresponding increase in blood volume increased the size of the heart slightly. That the increase in the latter case was not as great as one might have expected was probably due to the elasticity and distensibility of the vascular bed, which can take care of the increased blood volume without great dilatation of the heart.

6. Rous, P., and Turner, J. R.: A Rapid and Simple Method of Testing Donors for Transfusion, J. A. M. A. **64**:1980 (June 12) 1915.

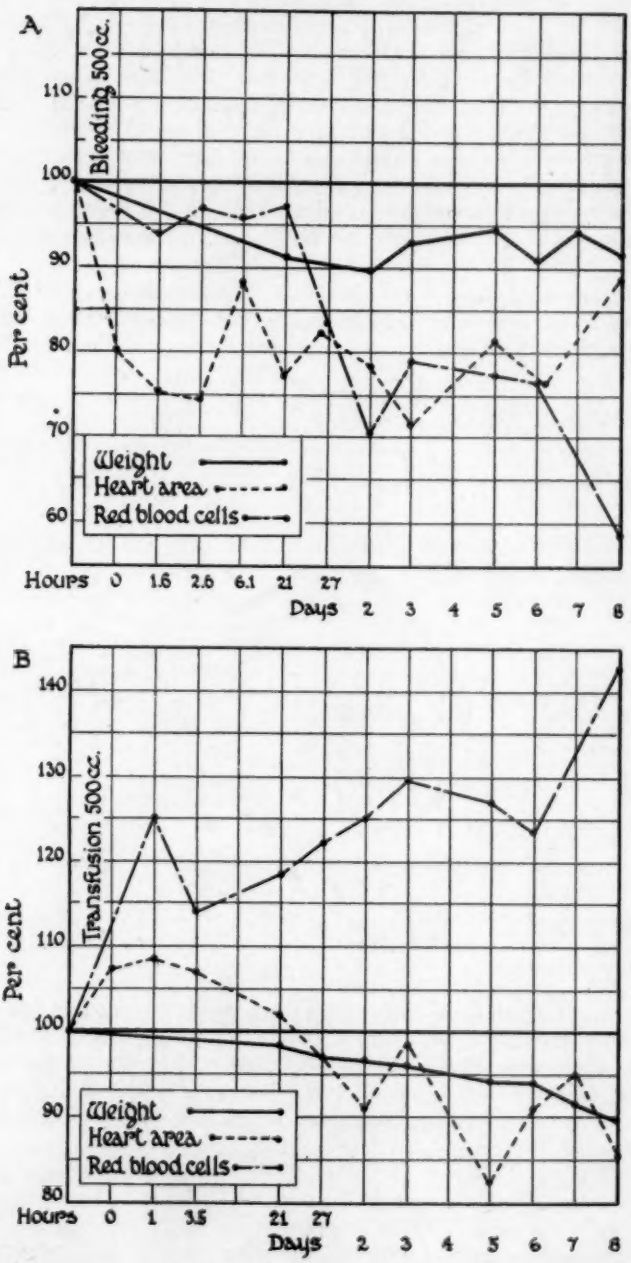

Fig. 8.—Graph *A* shows the effect of a decrease in blood volume on the cardiac area and the number of the red blood cells; graph *B*, the effect of an increase in the volume of the blood.

Effect of Diphtheria Toxin on the Regeneration of Red Blood Cells: Although counts of the red blood cells and studies of the blood volume do not point to destruction of blood as the cause of the decrease in cardiac size, jaundice seen in many of the dogs suggested that it might have occurred. In sections from some of the livers there were nests of cells[1] suggestive of foci of regenerating red blood cells, and, in some of these, cells resembling nucleated red blood cells were seen. It is unusual that signs of regeneration of red blood cells should appear so early after the injury, and, in addition, the liver is not commonly supposed to take on the function of formation of red blood cells until considerable anemia is present, or until damage has been suffered by the other blood-forming organs. The sections of the liver occasionally showed bile thrombi, but they were not present in the livers of all the animals exhibiting jaundice; therefore, this could not be the factor that caused jaundice. It is possible that jaundice was caused by destruction of red blood cells by the toxin; indeed, the destruction of a small amount of blood might readily have given rise to sufficient pigment

TABLE 9.—*The Effect of Increasing the Blood Volume by 500 Cc. on the Cardiac Area and the Number of Red Blood Cells (Dog 115)*

Time of Observation	Weight		Cardiac Area		Red Cells	
	Kg.	Per Cent of First Weight	Sq. Cm.	Per Cent of First Area	Millions	Per Cent of First Count
Before transfusion	11.96	100.0	47.55	100.0	6.4	100.0
Immediately after transfusion	.....	.....	51.35	107.9	...	.....
Hours after						
1	.....	.....	51.50	108.3	8.0	125.0
3.5	.....	.....	51.00	107.2	7.3	114.0
21	11.75	98.2	48.53	102.0	7.5	118.7
27	.....	....	46.30	97.3	7.8	121.8
Days after						
2	11.55	96.5	43.45	91.3	8.0	125.0
3	11.45	95.7	46.68	98.1	8.3	129.6
5	11.25	94.0	39.35	82.7	8.1	126.5
6	11.25	94.0	43.30	91.0	7.9	123.4
7	10.95	91.5	45.25	95.1	...	.....
8	10.75	89.8	40.60	85.4	9.1	142.5

to cause jaundice, although the decrease in the total number of red blood cells and the total blood volume was too small to be detected by the methods used.

Summary: Studies of the blood did not give evidence, therefore, that blood destruction was the factor concerned in the decrease in size of the heart in these animals. The presence of jaundice clinically, and of foci of cells in the microscopic sections of the organs after death which may have been regenerating red blood cells, however, suggests that a certain degree of blood destruction may have occurred.

Effect of Diphtheria Toxin on the Capillaries.—The possibility that diphtheria toxin is a poison to the capillaries was mentioned. The toxin may cause dilatation of the capillaries similar to that caused by histamine, and the total increase in the vascular bed so brought about would result in a redistribution of the blood in the body—in a sense, draining it away from the heart. The heart would then become smaller, although the amount of blood in the circulation was the same. That there was a change in the capillaries is suggested by the frequent occurrence of ecchymoses at autopsy.

Effect of Diphtheria Toxin on the Structure of the Heart Muscle.—The gross and microscopic examinations of the hearts of the dogs that died of intoxication with diphtheria toxin have already been reported.[1] Lesions of the fibers of the heart muscle or of the interstitial tissues that could be attributed to the diphtheria toxin were not found. Therefore, the decrease in cardiac area could not have been due to the actual destruction of muscle tissue.

Effect of Diphtheria Toxin on the Weight of the Heart Muscle.—There remains the possibility that the decrease in the size of the heart resulted from a loss of weight by the heart muscle itself. This was definitely exhibited in twelve dogs.

In dogs 81, 84, 86, 87, 97, 102, 103, 105, 107, 108, 110 and 112, there was a decrease in the ratio of the combined ventricular weights to the body weight (fig. 9B);[7] in these the heart muscle must have lost weight more rapidly than the body. If loss in both had proceeded at an equal pace, the ratio would have been undisturbed. In the other eight dogs, there might have been a decrease in heart weight, but in these the loss of weight by the body proceeded at a greater rate, so that the $\frac{L + R}{B W}$ ratio appeared greater than normal. This, however, did not preclude an absolute loss of weight by the heart muscle, although relative to the body weight this was not apparent. For this reason, the figures are misleading. This objection may be obviated in the following way: It may be assumed that, before the injection of the toxin, the weights of the hearts were such that $\frac{L + R}{B W}$ would have a value approximating the average for normal dogs. If the body weight observed just before the injection of the toxin is used to calculate $\frac{L + R}{B W}$, the weight of the heart at the time of death is seen to have decreased, so that in all animals, except four (dogs 82, 83, 99 and 104), the heart did not weigh as much as it should have for a dog as large as the animals were before the injection. There was a tendency for the $\frac{L + R}{B W}$ ratio to be lowest in those animals in which there was the greatest decrease in cardiac size (fig. 9B). The $\frac{L + R}{B W}$ ratios for all except the four dogs mentioned fell, then, below the average figure. These four dogs, however, showed large decreases in cardiac size and this mechanism cannot be called into play to explain them. The decrease in the L/R ratio (fig. 9A) in all dogs, except dog 109, showed that there was some disturbance in the heart muscle that caused the left side of the heart to lose weight more rapidly than the right side, resulting in a change in the ratio of the two sides. The decrease in the amplitude of the R_2 and R_3 waves (Stewart[1]) in the electrocardiogram also pointed to some disturbance in the muscle itself.

Summary: There was a decrease in the ratio of the combined ventricular weights to the body weight, which is most easily explained on the basis of a decrease in the weight of the heart. Parallel with this decrease in the weight of the heart, there was a decrease in the size of the heart. This relationship is still more striking, and the decrease in

7. The data from which figure 10 was constructed are contained in the first paper of this series.[1]

the weight of the heart can explain the decrease in size of the heart in all but four instances, if the ratio is calculated from the weight of the animal just before the injection of the toxin.

By what mechanism, then, does the heart muscle lose weight? Nothing in the microscopic sections of the heart muscle indicated that an actual destruction of heart muscle cells had taken place. One is forced to look for some other mechanism by which the heart muscle could lose weight. Since there was not any destruction of muscle cells, it is possible that a loss of substances, such, perhaps, as fluid and salts,

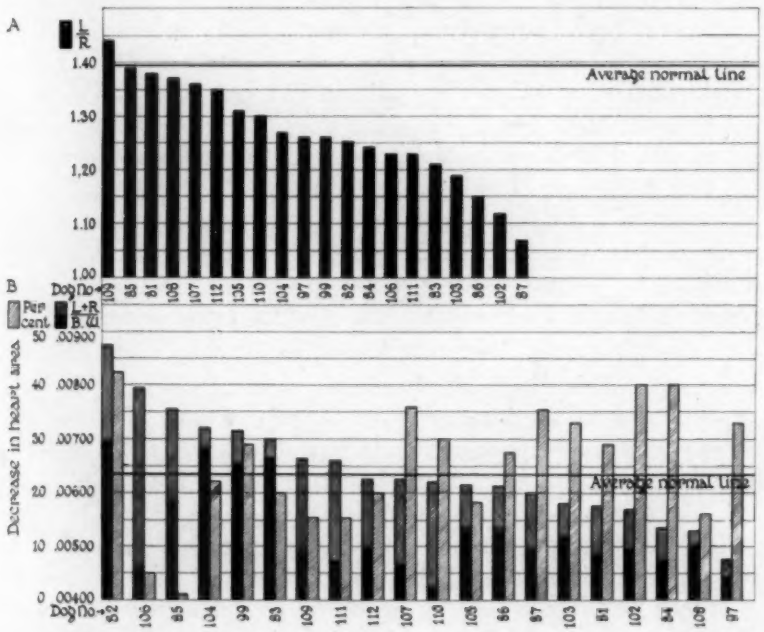

Fig. 9.—Graph *A* shows the grouping of the L/R ratios in dogs suffering from diphtheria intoxication with reference to the average L/R ratio in normal dogs. The height of the solid column represents the L/R ratio. Graph *B* shows the association between the change in the $\frac{L + R}{B W}$ ratio and the decrease in cardiac area in dogs suffering from diphtheria intoxication. The total height of the left hand column represents the $\frac{L + R}{B W}$ ratio when the weight of the dog at autopsy is used in calculating the ratio. The height of the solid column represents this ratio when the weight of the dog before the injection of diphtheria toxin is used in calculating this ratio. The height of the diagonally ruled area represents the decrease in cardiac area.

might account for the decrease in its weight and for the decrease in its size. Whether a change such as this actually occurred I cannot say; I am without evidence on this point.

COMMENT

Following the injection of 0.00168 cc. or more diphtheria toxin per kilogram of body weight into dogs, the cardiac area in the dogs, as measured in roentgenograms, decreased appreciably. It was found that 0.00135 cc. of toxin per kilogram of body weight caused a similar change, but that 0.001 cc. per kilogram did not. There was approximately the same decrease in body weight in all the dogs, an observation that precluded the possibility that the decrease in cardiac size was due to the decrease in body weight. Moreover, in dogs that had fasted for from three to four days, and in which there was a loss of body weight comparable to the loss of weight following diphtheria intoxication, there was no change in the size of the heart. This indicated that the decrease in cardiac size was due to the toxin. Counts of the red blood cells and estimations of the hemoglobin did not show evidence of marked destruction of blood. In these dogs, there was no consistent change in the total blood volume. In this connection, experiments showed that changes in blood volume are reflected in alterations in the size of the heart, in the following manner: A decrease in blood volume is accompanied by a decrease in the size of the heart and an increase in blood volume by a small increase in the size of the heart. The possibility that diphtheria toxin injures the capillaries was mentioned. A study of the $\frac{L + R}{B W}$ ratios, however, pointed to the conclusion that there was a loss of weight by the heart muscle which could account for a part of, if not for all, the decrease in the size of the heart. This mechanism failed to explain the phenomenon in four instances. Histologic study of the muscle of the heart did not reveal an actual destruction of the cells of this muscle to account for the decrease in weight. There is a possibility that the latter was due to a disturbance in some mechanism involved in the maintenance of the water balance of the heart muscle cells. It may be that the decrease in the weight of the heart is not the only factor causing the decrease in size of the heart; the other possibilities mentioned may also play a rôle. Whatever the mechanism, it must take place rapidly, for the decrease in cardiac size was always well developed within twenty-four hours and was often at a maximum at that time.

CONCLUSION

The injection of diphtheria toxin into a dog in a sufficiently large dose is followed by a decrease in the size of the heart. Analysis of the factors that may be involved in this alteration indicates that it is due to loss of weight by the heart, although other factors possibly play a part.

THE RETICULO-ENDOTHELIAL SYSTEM IN THE INFECTIOUS ANEMIA OF ALBINO RATS *

PAUL R. CANNON, M.D.
AND
PRESTON H. McCLELLAND, A.B.
CHICAGO

The importance of the reticulo-endothelial system as a mechanism of defense in infectious diseases is generally recognized, although there are differences of opinion as to the functions and modes of action of the individual cells. The significant fact is that the body is normally equipped with a system of mobile cells of mesodermal origin which respond to infectious agents by proliferation, by migration and by differentiation into more active forms. Maximow,[1] in his recent review, has discussed the most commonly accepted views as to the rôle of the mesenchymal reactions in the general mechanism of defense. The studies of Metchnikoff [2] on the comparative pathology of inflammation furnish a biologic background in the conception that, in the simpler forms of animal life, the ameboid mesodermal cells are the active agents of intracellular digestion. After the differentiation of the entoderm, this function assumes a less prominent rôle, but persists and may become active in time of stress. Metchnikoff also believed that the antibodies, although of secondary importance in immunity, were formed by these mesodermal cells, but merely as a consequence of their property of phagocytosis and intracellular digestion.

These views of Metchnikoff did not attain general acceptance for many years, although various workers contributed facts of importance regarding the activities of these cells in individual organs, such as the liver and the spleen. Morphologic studies with the so-called vital staining methods gradually led to the conception of a system of such phagocytic cells, and since Aschoff [3] and Landau [4] gave the name of

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* From the Department of Pathology and the Otho S. A. Sprague Memorial Institute, the University of Chicago.

1. Maximow, A. A.: The Morphology of the Mesenchymal Reactions, Arch. Path. **4**:557 (Oct.) 1927.

2. Metchnikoff, E.: Leçons sur la pathologie comparée de l'inflammation, Paris, Masson & Cie, 1892.

3. Aschoff, Ludwig: Das reticulo-endotheliale System, Ergebn. d. inn. Med. u. Kinderh. **26**:1, 1924.

4. Landau, M.: Ber. d. Naturforsch. Gesellsch. zu Freidburg, 1913, vol. 20; cited by Aschoff (footnote 3).

reticulo-endothelial system to this aggregation of cells, interest has been intensified in the activities of the system as a whole. Previously, the suggestion had been made that these cells, particularly in the spleen and the liver, were the active agents in the normal destruction and digestion of erythrocytes, and Kyes [5] named them hemophages with that idea in mind. Motohashi [6] later showed that in the rabbit this function is performed mainly in the spleen, to be assumed by the Kupffer cells of the liver and the macrophages of the bone marrow after the spleen is removed. It is probable that the phagocytosis of bacteria is merely a further adaptation of this general function of ingestion of particulate matter of various kinds.

In the present paper, we shall consider the reticulo-endothelial system as consisting, in general, of all of the mesodermal cells of the body which are or may become endowed with the property of phagocytosis to a high degree. Although these cells are concentrated to the most marked extent in the spleen, liver, bone marrow, omentum, lymph nodes and lungs, they are probably distributed throughout all the connective tissues of the body. As Maximow has shown the importance of the lymphocyte as a potential macrophage, the entire lymphatic system should be considered as the biologic foundation of the reticulo-endothelial system.

Of all the individual organs of this system, the spleen ranks first, in mammals, as the organ richest in histiocytes. But even here, there appear to be differences between species as to the relative amount of reticulo-endothelial tissue in the spleen. Krumbhaar [7] has recently stated that the ratio of the weight of the spleen to that of the body indicates that the spleen is relatively of more significance in man, the dog and the rat, of less in the monkey and guinea-pig and of least in the cat and rabbit (fig. 1). It is suggestive that most of the earlier work on the effects of splenectomy on the production of antibodies was done with rabbits and guinea-pigs, animals in which the spleen, quantitatively, is of least significance. The conflicting results may well be explained, in part, by this. It would appear that in the study of the relative importance of the histiocytic functions of the spleen, the dog and the rat are better for use in experiments than are the monkey, the guinea-pig and the rabbit. As a matter of fact, the most convincing experiments as to the rôle of the spleen in the production of antibodies and in resistance to infection have been performed with the dog and the rat

5. Kyes, Preston: Internat. Monatschr. f. Anat. u. Physiol. **31**:543, 1914.

6. Motohashi, Shinzo: The Effect of Splenectomy on the Production of Antibodies, J. M. Research **43**:473, 1922.

7. Krumbhaar, E. B.: Functions of the Spleen (Mysterii Plenum Organon) Galen, Physiol. Rev. **6**:160, 1926.

(Bardach,[8] Hektoen,[9] Luckhardt and Becht,[10] Morris and Bullock,[11] Kritschewski and Rubinstein,[12] Lauda [13] et al). For example, Luckhardt and Becht found that splenectomized dogs did not produce hemolysins, hemagglutinins or hemopsonins as rapidly nor in as high concentration as normal dogs; and Hektoen, using rats, found the production of hemolysin markedly lowered after splenectomy. Bardach, in his work on anthrax, found that splenectomy in dogs led to a significantly higher fatality rate than was the case with normal animals. Morris and Bullock observed that the fatality rate in a large series of

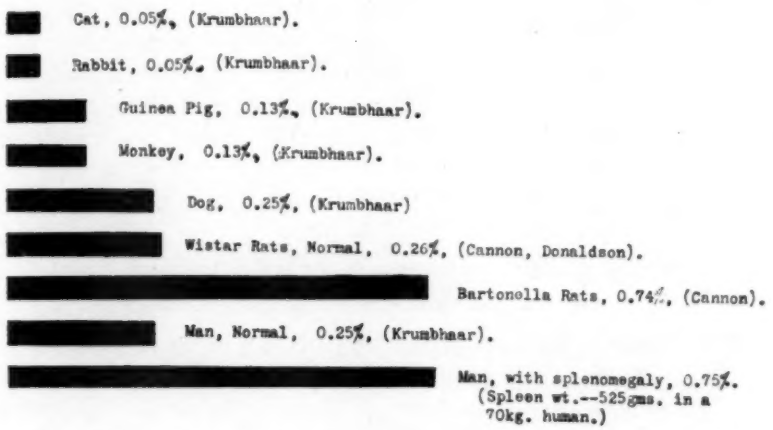

Fig. 1.—The relative ratios of the weight of the spleen to that of the body in various species, with comparison of the ratio for normal rats with that for Bartonella-infected rats and of the ratio for normal man with that of man with splenomegaly.

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8. Bardach, M. J.: Recherches sur le rôle de la rate dans les maladies infectieuses, Ann. de l'Inst. Pasteur **3**:577, 1889.

9. Hektoen, Ludvig: Further Observations of the Effects of Roentgenization and Splenectomy on Antibody Formation, J. Infect. Dis. **27**:23, 1920.

10. Luckhardt, A. B., and Becht, F. C.: The Relation of the Spleen to the Fixation of Antigens and the Production of Immune Bodies, Am. J. Physiol. **28**:257, 1911.

11. Morris, D. H., and Bullock, F. D.: The Importance of the Spleen in Resistance to Infection, Ann. Surg. **70**:513, 1919.

12. Kritschewski, J. L., and Rubinstein, P. L.: Immunity in Relapsing Fever: 1. The Influence of the Reticulo-Endothelial System, Ztschr. f. Immunitätsforsch. u. exper. Therap. **51**:27, 1927.

13. Lauda, E.: Ueber die bei Ratten nach Entmilzung auftretenden schweren anämischen Zustände. "Perniciöse Anämie der Ratten," Virchows Arch. f. path. Anat. **258**:529, 1925.

splenectomized rats exposed to chance infection with so-called rat plague was much greater than with orchidectomized animals kept under similar conditions. These results indicate that in the case of animals in which there is a comparatively large amount of reticulo-endothelial tissue in the spleen, the removal of this organ leads to decreased production of antibodies and to decreased resistance to certain bacterial infections.

The demonstration that particulate matter and certain colloidal substances are selectively removed from the circulation led to the idea of saturating or "blocking" the cells of the histiocytic system, thereby reducing to a minimum their further powers of phagocytosis and of production of antibodies. Numerous attempts to produce this condition have been made, using particularly india ink and trypan blue, but here again the results have been extremely variable. In some instances, this variability has undoubtedly been due to an insufficient amount of the material injected; in others, to a failure to maintain the saturation during the period of observation. The positive results of Murata,[14] Gay and Clark,[15] Stewart and Parker,[16] Bieling and Isaac,[17] Siegmund[18] and others, however, point strongly to the conclusion that a blockade under proper conditions materially depresses the production of antibodies.

THE PART PLAYED BY THE SPLEEN IN THE DEFENSE MECHANISM OF THE RETICULO-ENDOTHELIAL SYSTEM

More recently, the method has been applied, particularly in conjunction with splenectomy, to animals infected with various organisms. Thus, Kritschewski and Rubinstein[12] found that, whereas the fatality rate in normal mice infected with *Spironema duttoni* is 3.82 per cent, in mice splenectomized three days after the infection the rate is 82.15 per cent; and in splenectomized mice into which a solution of iron saccharate has been injected to blockade the remainder of the reticulo-endothelial system, the death rate is 90.47 per cent. The

14. Murata, M., quoted by Aschoff: Lectures on Pathology, New York, Paul B. Hoeber, 1924, p. 28.

15. Gay, F. P., and Clark, A. R.: The Reticulo-Endothelial System in Relation to Antibody Formation, Proc. Soc. Exper. Biol. & Med. **22**:1, 1924; also J. A. M. A. **83**:1296 (Oct. 25) 1924.

16. Stewart, F. W., and Parker, Frederick, Jr.: So-Called "Endothelial Blockade" with Collargol. An Immunologic and Histologic Study, Am. J. Path. **2**:381, 1926.

17. Bieling, R., and Isaac, S.: Experimentelle Untersuchungen über intravitale Hämolyse: IV. Die Bedeutung des Reticulo-Endothels, Ztschr. f. d. ges. exper. Med. **28**:180, 1922.

18. Siegmund, H.: Speicherung durch Reticuloendothelien, celluläre Reaktion und Immunität, Klin. Wchnschr. **1**:2566, 1922.

apparent lack of the formation of antibodies in relapsing fever in mice after blockade and splenectomy was demonstrated by Jungeblut.[19]

In view of these facts, the so-called *Bartonella* infection of rats offers an unusual opportunity to study the functions of the reticuloendothelial system as a whole by blockade methods, and in part, by splenectomy. Furthermore, the remarkable latency of this infection ensures the utilization of an animal naturally infected.

Observations on the Effect of Splenectomy in Bartonella-Infected Rats.—The disease due to *Bartonella muris* was first described by Lauda[18] as the infectious anemia of rats, occurring only after the removal of the spleen. In our laboratory, splenectomy in a large series of infected rats has invariably led to a rapidly developing anemia, usually within from five to nine days, which is frequently accompanied by hemoglobinuria. Coincidently, the small coccobacillary bodies known as *Bartonella* organisms are to be seen on the erythrocytes after the staining with Giemsa stain. The surprising feature is that animals with this so-called *Bartonella* infection appear healthy, and the disease flares up only after removal of the spleen. Removal of one or both suprarenal glands, one or both testes, the thyroid gland, the omentum, or both cerebrums of the brain does not have this effect; in these same animals, splenectomy is followed by the development of the typical anemia.[20] Removal of from one third to one half of the spleen does not have any effect; removal of two thirds of the organ sometimes is followed by a milder grade of anemia. When the pedicle of the spleen is ligated and the organ excised and placed in the general peritoneal cavity, the anemia develops as with splenectomy, indicating the necessity of an intact blood supply to keep the virus under control.

Observations on the Size of the Spleen and Its Histologic Character in Bartonella-Infected Rats.—A fact of great significance is the size of the spleen in infected animals as compared with normal, uninfected ones. We weighed the spleen at operation in eighty-eight rats infected with the *Bartonella* virus. The average ratio of the weight of the spleen to that of the body for these was 0.74 per cent. As shown in figure 1, this corresponds to a spleen weighing 525 Gm. in a human being weighing 70 Kg. In contrast with these figures, we have the average found by Donaldson[21] for eighty-seven normal rats: 0.26 per cent. Our figures for thirty-four splenectomized Wistar rats, ranging in age from 3 weeks

19. Jungeblut, C. W.: Ueber die Beziehungen zwischen retikuloendothelialen System und chemo-therapeutischer Wirkung, Ztschr. f. Hyg. u. Infectionskrankh. **107**:357 1927.

20. Cannon, P. R.; Taliaferro, William, and Dragstedt, L. R.: Anemia Following Splenectomy in White Rats, Proc. Soc. Exper. Biol. & Med. **25**:359, 1928.

21. Donaldson, H. H.: The Rat. Data and Reference Tables. Memoirs of the Wistar Institute of Anatomy and Biology, no. 6, Philadelphia, 1924.

to 18 months, give an average of 0.25 per cent. The difference between the ratios of the weight of the spleen to that of the body in *Bartonella*-infected rats and normal, uninfected Wistar rats is so striking that we feel that it is frequently possible to determine by this feature alone whether or not the animal has had the *Bartonella* infection.

Histologically, we find indubitable evidence of the reactive tendency of the reticulo-endothelium of the spleen in the presence of the *Bartonella* virus. Spleens from normal, uninfected Wistar strain rats

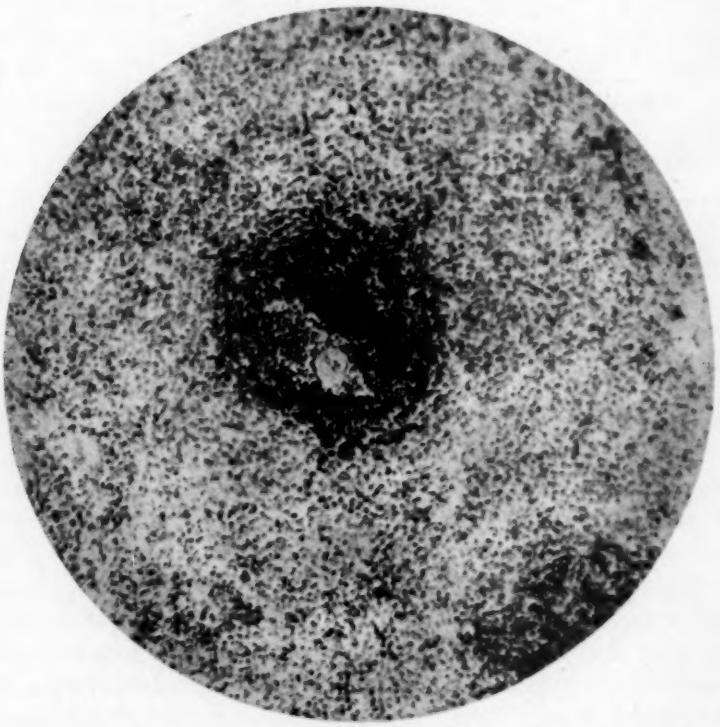

Fig. 2.—Photomicrograph (× 170) of a splenic follicle in a normal rat. Note the compact collection of lymphocytes with a relatively narrow marginal zone.

are small and firm; the splenic follicles are compact, composed mainly of lymphocytes with only moderate evidence of hyperplasia of the centers of the follicles, and they have a relatively narrow marginal zone. On the other hand, spleens taken at operation from *Bartonella*-infected animals are large and fleshy; microscopically, the splenic follicles are large with marked evidence of activity in the centers, as shown by numerous mitotic figures, and are surrounded by a broad marginal zone also containing many mitotic figures and hyperchromatic nuclei.

The picture is that of distinct hyperactivity and even hyperplasia of the reticulo-endothelial elements (figs. 2, 3 and 4).

There can be little question, therefore, that the spleen acts in some manner to keep the *Bartonella* virus under control as a latent infection. When this inhibitive influence is removed, the virus apparently develops rapidly with the concomitant acute anemia. It is conceivable that the spleen, as an important part of the reticulo-endothelial system, may exert its controlling effect either by phagocytosis of the *Bartonella* virus

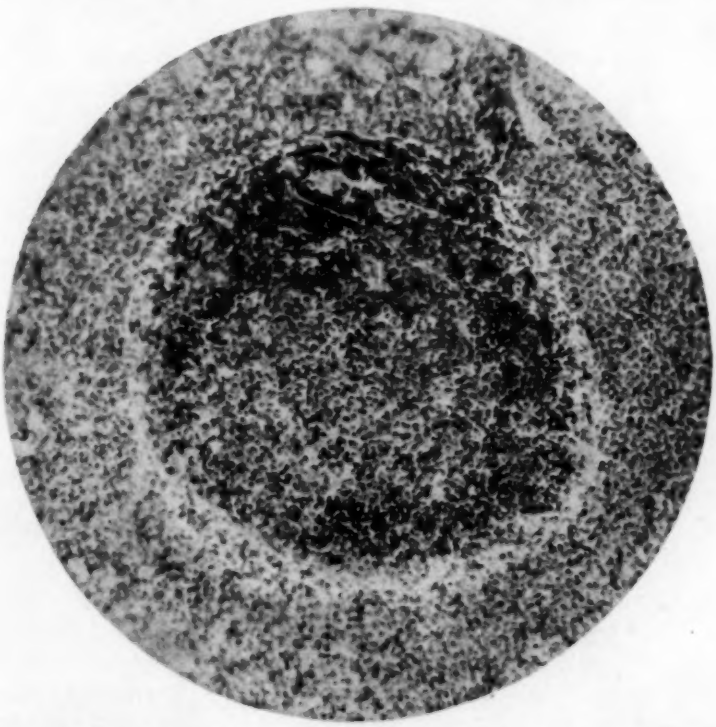

Fig. 3.—Photomicrograph (× 170) of a splenic follicle of a Bartonella-infected rat of the same age as the one shown in figure 2. Note the enlarged follicle with a prominent germinal center and a broad marginal zone.

at a constant rate or by the formation of antibodies of some type which restrain the development of or destroy the organisms or by both these processes. In any case, when the balance is disturbed between the offensive powers of the organisms and the defensive mechanisms of the host, the infection flares up and the anemia results. It is evident, then, that the "factor of safety" in the resistance to this infection resides in the spleen, equalling approximately two thirds of this organ; the removal of this factor allows the disease to progress.

EXPERIMENTS IN BLOCKADING THE RECTICULO-ENDOTHELIAL SYSTEM OF SPLENECTOMIZED AND NONSPLENECTOMIZED RATS INFECTED WITH BARTONELLA MURIS

If the latency of this infection is the result of the defensive activities of the reticulo-endothelial system, a saturation of the cells of this system with particulate matter to as high a degree as possible should enable the *Bartonella* virus to gain the ascendancy with a resulting anemia and the reappearance of *Bartonella* bodies on the erythrocytes. Further-

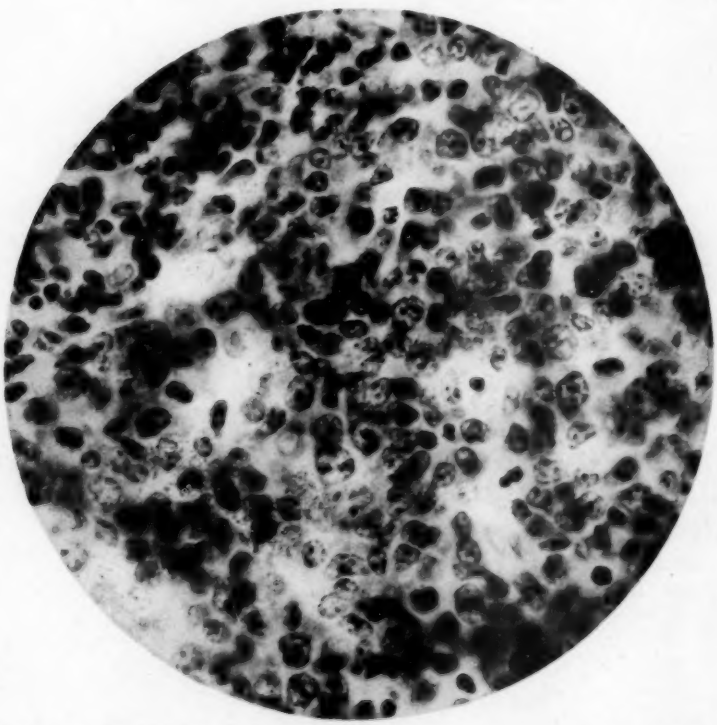

Fig. 4.—Photomicrograph (× 650) of the germinal center of the splenic follicles shown in figure 3. Numerous mitotic figures and hyperchromatic nuclei may be seen.

more, splenectomized animals that have recovered from the anemia should nevertheless have a lower factor of safety and thus should suffer a relapse after a blockading of the remaining elements of the histiocytic system.

Method.—With that thought in mind, Higgin's india ink was prepared as a 4 per cent suspension in 0.85 per cent sodium chloride solution, filtered two or three times through no. 595 filter paper (Schleicher and Schüll) and autoclaved. This was then injected intraperitoneally, in 10 cc. amounts, at daily intervals, into three splenectomized *Bartonella* infected rats and three rats of the Wistar strain.

All had been splenectomized from one to two months previously, the former having had the typical infectious anemia and having returned to an approximately normal condition so far as the blood picture was concerned.

Series 1, Using Splenectomized Rats.—The injection of a total of 80 cc. of the india ink led, in the case of *Bartonella*-infected rats, nos. 111T, 112T and 130T, to a marked drop in the hemoglobin concomitantly with the reappearance of countless *Bartonella* bodies on the erythrocytes at the height of the anemia. The injection of the india ink into the splenectomized animals of the Wistar strain (nos. 136T, 140W and 141W) did not have this effect, there being only a slight drop in hemoglobin values, although a larger total amount of ink was injected. Figure 5 illustrates the course of events in this series, and table 1 gives the amount of ink injected per gram of body weight for each animal. The moving average method has been used in figures 5, 6 and 7.

TABLE 1.—*Results of Blockading the Reticulo-Endothelium System of Splenectomized Bartonella-Infected Rats*

Rats	Amount of Ink Injected, in Cc. of 4% Suspension, per Gm. Body Weight	Effect
111T	0.27	Anemia
130T Bartonella-infected rats	0.51	Anemia
112T	0.21	Anemia
136T	1.06	No anemia
140T Normal controls	0.82	No anemia
141T	0.97	No anemia

Giemsa stains showed the blood picture in the uninfected animals to be essentially normal throughout the period of observation.

The experiments of Nagao [22] and Brickner,[23] using injections of india ink, showed that this material is taken up to a marked degree by the histiocytes of the reticulo-endothelial system. Presumably, in series 1, the phagocytosis of the ink particles by these cells so depressed their activities toward the *Bartonella* infection that a relapse occurred; at least, the blood picture was of the same type as that following splenectomy in these animals.

Series 2, Using Nonsplenectomized Rats.—We next repeated the experiment, using four infected rats (nos. 105T, 106T, 108T and 109T) the spleens of which had not been removed. The daily intraperitoneal injection, in 10 cc. amounts, of a 4 per cent suspension of india ink had only a slight effect, even after prolonged treatment. In rat 105T, there was a decline in hemoglobin from an initial value of 12.5 Gm. per hun-

22. Nagao, K.: The Fate of India Ink Injected into the Blood: I. General Observations, J. Infect. Dis. **27**:527, 1920.

23. Brickner, R. M.: The Rôle of the Capillaries and Their Endothelium in the Distribution of Colloidal Carbon by the Blood Stream, Bull. Johns Hopkins Hosp. **40**:90, 1927.

dred cubic centimeters of blood to 7.9 Gm., together with a few suspicious looking *Bartonella* bodies on the erythrocytes. The continuance of the injections until 240 cc. of the ink suspension had been injected did not cause the hemoglobin to go any lower. Similar results were obtained with rat 106T. After 170 cc. of ink had been injected intraperitoneally, the hemoglobin value had fallen from 13.5 Gm. per hundred cubic centimeters of blood to 7.9 Gm. and there was marked polychromatophilia, anisocytosis and an occasional normoblast in the blood smear. At no time, however, were definite bartonellas seen.

These results suggested that in order to get a "functional paralysis" of the reticulo-endothelial cells, one must saturate them to a maximal degree in a minimum of time in order to prevent proliferative activities of the histiocytes from interfering with the course of events. With this end in view, rats 108T and 109T were given injections intraperitoneally

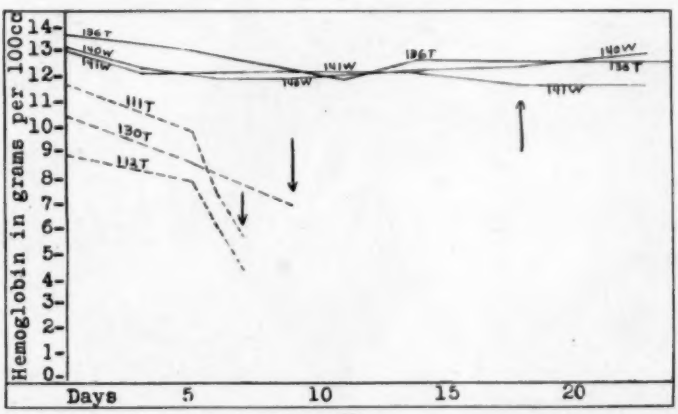

Fig. 5.—The straight line represents splenectomized Wistar rats; the broken line, Bartonella-infected rats. The graphs show the hemoglobin curves for three splenectomized Bartonella-infected rats that were given daily injections of a 4 per cent suspension of Higgin's india ink in 0.85 per cent sodium chloride solution. Injections were begun on the first day and were discontinued on the days indicated by the arrows.

of 60 cc. of a 4 per cent suspension of india ink in sodium chloride solution, in 10 cc. amounts twice daily, followed by daily intravenous injections of from 5 to 6 cc. of the suspension of ink, for eleven days. During this period, the hemoglobin of rat 108T fell from 13.5 Gm. per hundred cubic centimeters of blood to 5.2 Gm., with a rapidly increasing polychromatophilia and anisocytosis. At the lower reading, normoblasts appeared but definite bartonellas were not seen. The hemoglobin content of rat 109 T dropped from 15.1 Gm. per hundred cubic centimeters of blood to 7.6 Gm., but here again, definite bartonellas were not seen. Figure 6 shows the hemoglobin trend for these animals, and table 2 gives the amount of the suspension of ink injected per gram of body weight.

It is interesting that the anemia was more marked in the animals getting the intravenous injections, although much less of the suspension of ink per gram of body weight was injected.

Series 3, with Maximal Saturation of Reticulo-Endothelial Systems of Bartonella-Infected Rats in Minimal Time.—The results of series 2 indicated clearly that an effective blockade of the histiocytic system demands maximal saturation in minimal time. Previous workers found that small amounts of injected material may stimulate rather than depress the activities of these cells. Furthermore, there is evidence that, under this stimulation, an actual hyperplasia of the reticulo-endothelial system occurs. If the histiocytes secrete a substance that restrains the

TABLE 2.—*Results of Prolonged Injection of India Ink into Nonsplenectomized, Bartonella-Infected Rats Compared with Results of Maximal Saturation in Minimal Time*

Rat	Amount of Ink Injected, in Cc. of 4% Suspension, per Gm. Body Weight	Effect
105T	1.34 (prolonged period)	Moderate anemia
106T	1.44 (prolonged period)	Moderate anemia
108T	0.80 (minimal period)	Moderate anemia
109T	0.75 (minimal period)	Moderate anemia

TABLE 3.—*Results of Saturating the Reticulo-Endothelial Systems of Bartonella-Infected Rats with Intravenous Injections of India Ink*

Rat	Amount of Ink Injected, in Cc. of 8% Suspension, per Gm. Body Weight	Effect
162 (normal control)	0.68	No anemia
132	0.63	Moderate anemia
163	0.65	Marked anemia
164	0.69	Marked anemia

development of the activities of the *Bartonella* virus, it is imperative that these cells shall be completely saturated and kept in a state approaching saturation.

Therefore, an 8 per cent suspension of Higgin's india ink in 0.85 per cent sodium chloride solution was prepared as outlined heretofore. Three *Bartonella*-infected rats, nos. 132, 163 and 184, were given injections intravenously, twice daily, of from 5 to 6 cc. of the suspension of ink. At the same time Wistar strain rat 162 was similarly treated as a control. Figure 7 shows the hemoglobin curves and table 3 the amount of ink injected per gram of body weight.

The most significant feature in this series was the demonstration of the relatively enormous amount of the suspension of ink necessary to cause the severe anemia to appear. It is evident, then, that adequate blockade of the reticulo-endothelial system requires the intensive intravenous and intraperitoneal injection of large amounts of the blockading

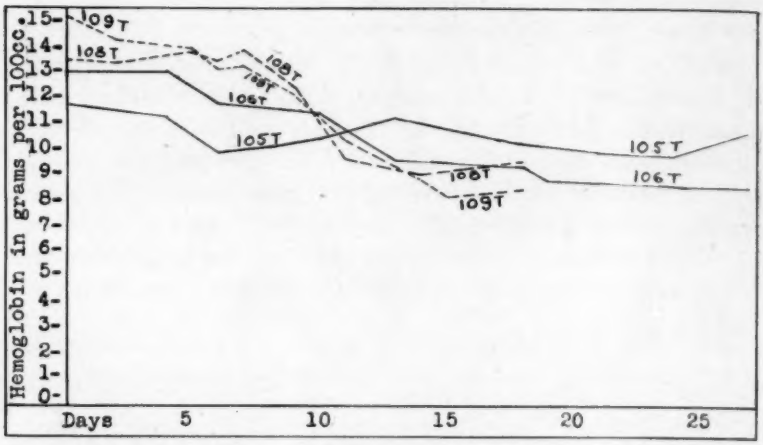

Fig. 6.—The graphs show the hemogobin curves for four Bartonella-infected rats that were given injections of a 4 per cent suspension of Higgins' india ink in 0.85 per cent sodium chloride solution.

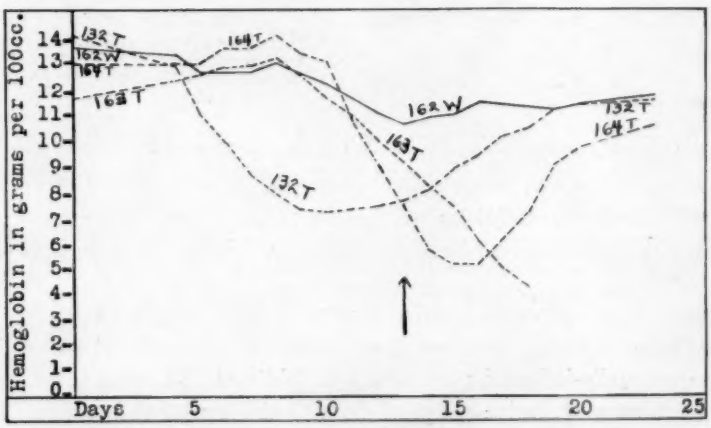

Fig. 7.—The straight line represents normal Wistar rats; the broken line, Bartonella-infected rats. The graphs show the hemoglobin curves for one normal Wistar rat and three Bartonella-infected rats that twice daily were given injections of an 8 per cent suspension of Higgins' india ink in 0.85 per cent sodium chloride solution. Injections were begun the first day, and were discontinued on the day indicated by the arrows.

material over a period of several days; in our series it took at least two weeks.

COMMENT

The spleen has long been considered a mysterious organ; yet accumulating evidence consistently indicates its importance in the defensive struggle against bacterial agents. The splenic swelling in many of the acute and chronic diseases of bacterial and protozoan origin confirms this. This enlargement was thought by Goldzieher [24] to be due to an actual hyperplasia of the reticulo-endothelial elements of the spleen, being especially evident in typhoid fever, acute sepsis and malaria. He interpreted this as a defensive reaction of a purposive nature.

The statement has been frequently made, even in recent literature, that the spleen is not essential to life. The significant question is: Is the spleen essential to life when the host is subjected to undue hazards? In other words, does not this organ represent a large "factor of safety" which, under abnormal conditions, may be the determining factor in resistance to infection? At such a time, splenectomy may decide the contest in favor of the infecting organism. This point has been particularly stressed by Morris and Bullock [11] and by Kikuth.[25] The recent review of splenectomies at the Mayo Clinic [26] indicates the same probability in conditions of acute infection.

Of wider biologic significance, however, is the rôle of the reticulo-endothelial system as a whole, of which the spleen is only a part. It would appear in view of the studies of Gay [27] and his students that the cells of this system are of more importance in streptococcus infections than was formerly believed to be the case. Without necessarily minimizing the value of the microphage as a cell of defense, the macrophage now assumes more significance.

The function of phagocytosis, possesssed to a marked degree by the histiocytes, led to the attempts to "blockade" this system which have given such variable results. It is of interest that Bardach,[8] as early as 1889, injected a suspension of wood charcoal into dogs and then, later, anthrax bacilli, and found that the animals all succumbed to the infection in spite of the marked insusceptibility of the dog to anthrax. Although the more recent attempts to influence the production of antibodies have been relatively inconclusive, it must be remembered that there is a vast difference in the modes of procedure. For instance, it

24. Goldzieher, M. A.: The Structure of Infectious Splenic Swelling, Arch. Path. **3**:42 (Jan.) 1927.

25. Kikuth, Walter: Studien zur Bedeutung der Milz als Abwehrorgan bei Infektionskrankheiten, Klin. Wchnschr. **6**:406, 1927.

26. Mayo, W. J.: A Review of 500 Splenectomies with Special Reference to Mortality and End Results, Ann. Surg. **88**:409, 1928.

27. Gay, F. P., and Morrison, L. F.: Studies in Streptococcus Infection and Immunity: V. Clasmatocytes and Resistance to Streptococcus Infection, J. Infect. Dis. **33**:338, 1923.

cannot be merely assumed that the system is "blocked"; histologic examinations must be made in order to prove it. The injections should be made intravenously, for, as we have found, daily intraperitoneal injections of relatively enormous amounts of a suspension of india ink over a period of thirty days may result in but minimal quantities of ink in the bone-marrow. It is probable that these intraperitoneal injections soon lead to a proliferation of the peritoneal mesothelium, so that little of the ink injected later is absorbed. This may explain why, after such injections into the *Bartonella*-infected animals, the maximal anemia appeared within a few days, followed by a gradual improvement in spite of the continued daily injections of the suspension of ink.

These observations may be of significance in connection with some other conditions in which there is evidence of hyperactivity and hyperplasia of the spleen. For instance, splenectomy in pernicious anemia was suggested by Eppinger on the hypothesis that there is a "hypersplenism" of unknown cause. It is more probable, however, that in most of the infections and anemias in which the spleen is enlarged this enlargement is secondary rather than primary. In the present instance, we have physiologic and morphologic evidence that the hyperactivity and hyperplasia of the reticulo-endothelial elements of the spleen are compensatory mechanisms of defense, the splenomegaly thus being the consequence rather than the cause. Removal of this mechanism allows the virus to develop relatively without restraint and the infectious anemia results.

SUMMARY AND CONCLUSIONS

The removal of the spleen in albino rats infected with the virus of so-called *Bartonella muris* leads to the development of an acute anemia, the infectious anemia of rats.

The removal of such other organs as the suprarenal glands, the testes, the omentum, the thyroid gland or the cerebrums does not have this effect. In these same animals, the later removal of the spleen causes the anemia to appear.

The enlarged spleens in such animals are the result of hyperactivity and hyperplasia of the reticulo-endothelial elements, this being a compensatory mechanism of defense.

Adequate blockade of the entire reticulo-endothelial system with india ink in *Bartonella*-infected rats is followed by the development of an anemia of the same type as that following splenectomy.

The reticulo-endothelial system, in general, and the spleen, in particular, restrain the development of the *Bartonella* virus and establish the latency of the infection; interference with this system, either by ablation or by saturation of the phagocytic cells with particulate matter, allows the virus to develop unduly with the resultant infectious anemia.

The "factor of safety" in this defensive mechanism is in the spleen; the removal of this factor is followed by a recurrence of the infection.

SICKLE CELL ANEMIA

FURTHER INVESTIGATION OF A CASE OF SPLENIC ATROPHY WITH CALCIUM AND IRON INCRUSTATIONS (NODULAR SPLENIC ATROPHY) *

G. A. BENNETT, M.D.
BOSTON

In a recent publication [1] I reported observations, from the necropsy material of a negress, aged 20, on an unusual spleen which weighed 10 Gm. and which showed microscopically the presence of calcium and iron incrustations in the walls of blood vessels and in the fibrous and elastic tissues of the trabeculae. No relationship between the atrophic spleen and the patient's acute illness seemed warranted, though her death during the engorgement stage of a bilateral lobar pneumonia seemed most unusual. The following conclusions were drawn in commenting on the possibilities in the previous report: "It seems that because of the iron in isolated fibers and in areas of considerable size without the presence of calcium the iron was of primary importance. It also seems that there was some etiologic factor, either in hemorrhage or in necrosis, or possibly in both, to cause the deposition of such a large amount of iron."

My attention has since been called to the possibility that the entire splenic condition might have been explained on the basis of sickle cell anemia.[2] For this reason the necropsy material and the clinical record were reexamined. Examination of the blood during life showed a hemoglobin value of 65 per cent (Tallqvist); red cells 3,040,000 and white cells 38,000 per cubic millimeter. The stained smear showed variation in size and shape with some polychromatophilia and fragmentation of the red cells. There were four reticulated cells seen. The differential count was: polymorphonuclears, 87 per cent; large mononuclears, 7 per cent; lymphocytes, 5 per cent, and basophils, 1 per cent. Smear preparations made from intravascular blood clots of tissues fixed in 10 per cent

* Submitted for publication, March 28, 1929.

* From the Department of Pathology of the Peter Bent Brigham Hospital and Harvard Medical School.

1. Bennett, G. A.: Splenic Atrophy with Calcium and Iron Incrustations (Nodular Splenic Atrophy), Arch. Path. **7**:71 (Jan.) 1929.

2. Bernhard Steinberg, M.D., Toledo, Ohio, and G. S. Graham, M.D., Birmingham, Ala.

formaldehyde revealed nearly 100 per cent sickle cells (fig. 1), while similarly prepared and treated smears from control cases showed red blood cells of normal contour (fig. 2). Graham [3] and later Hahn and Gillispie [4] observed that sickle cell distortion could be seen in sections of formaldehyde hardened tissues from affected subjects, whereas the red blood cells were not sickled in tissues fixed in Zenker's fluid.

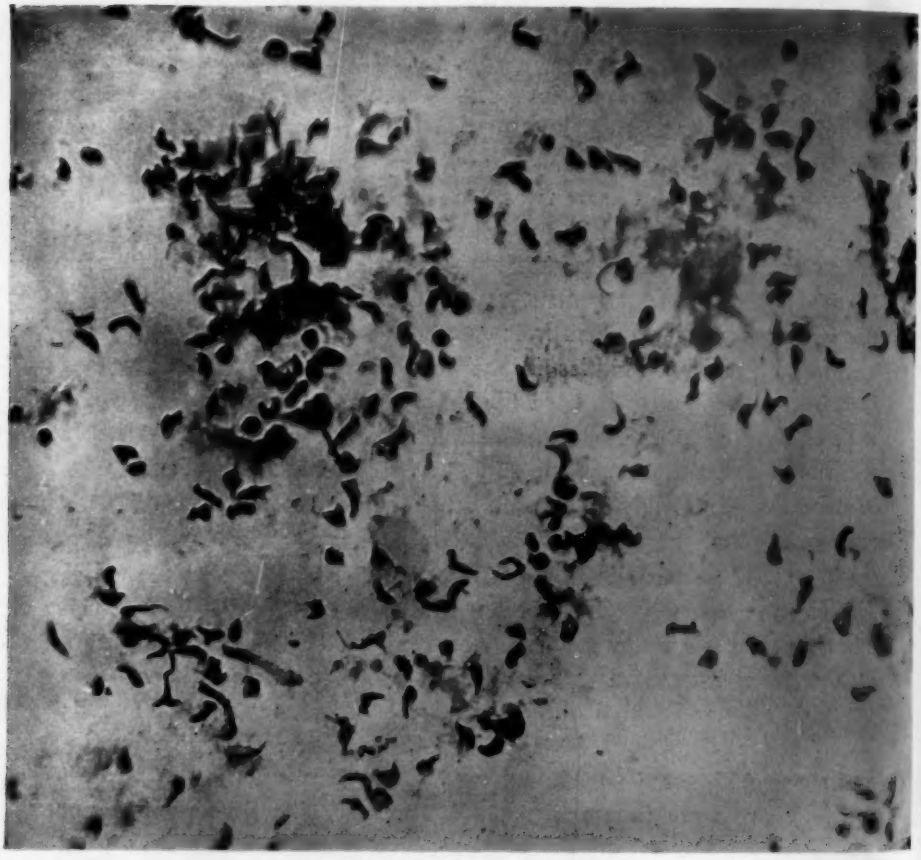

Fig. 1.—Nearly complete sickle cell distortion as seen in smears from blood clot fixed in formaldehyde.

It is worth while noting that a recent examination of the blood of the mother, who is the only living relative, showed none of the sickle cell traits, and there was no anemia.

3. Graham, G. S.: A Case of Sickle Cell Anemia with Necropsy, Arch. Int. Med. **34**:778 (Dec.) 1924.

4. Hahn, E. V., and Gillispie, E. B.: Sickle Cell Anemia, Arch. Int. Med. **39**:233 (Feb.) 1927.

SUMMARY

The case previously reported because of an unusual atrophic spleen with calcium and iron incrustations should undoubtedly be regarded as a case of sickle cell anemia.

Sickle cell anemia may be recognized from necropsy material by crushing blood clot from tissues hardened in 10 per cent formaldehyde in physiologic solution of sodium chloride and making smear preparations from the resultant suspension. The elapse of time after fixation seems to be of no importance in the amount or character of the red cell distortion.

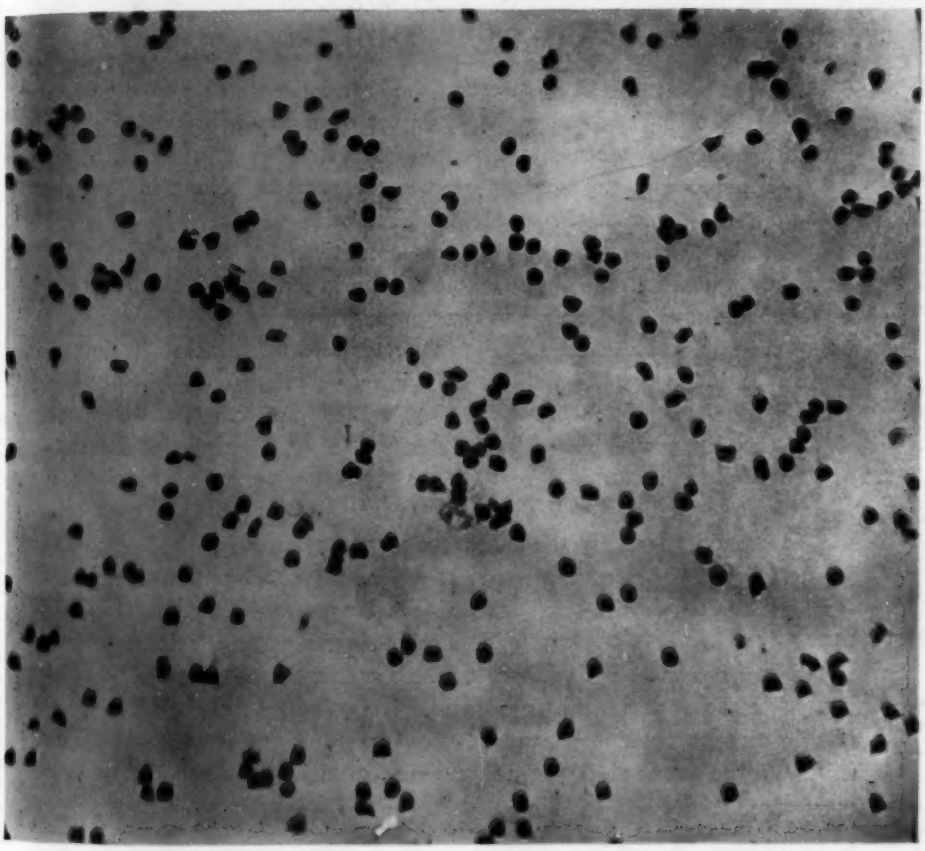

Fig. 2.—Red blood cells as seen in control smears from unaffected subjects. Note the preservation of the cell shapes after two years' fixation in 10 per cent formaldehyde. Method of preparation of figures 1 and 2 was identical.

THE EFFECT ON THE WHITE BLOOD CELLS OF INTRAPERITONEAL INJECTION OF WHOLE LIVER*

M. M. HUFFMAN, M.D.

JOHN S. LAWRENCE, M.D.

AND

EDGAR JONES, A.B.

NASHVILLE, TENN.

Previous observations have established a relationship between certain forms of hepatic disorder and the number of mononuclear cells in the circulating blood. Jones and Minot [1] observed both a relative and an absolute increase in the number of monocytes in the blood in patients with catarrhal jaundice. More recently, Thewlis and Middleton [2] studied the blood in thirty-eight cases of this condition and described similar changes. The essential observations of the latter investigators were a leukopenia, a marked decrease in the number of polymorphonuclear cells, a moderate decrease in that of lymphocytes and an increase in that of monocytes. A somewhat similar observation was reported by Minot and Smith [3] for occupational tetrachlorethane poisoning, in which there was an increase in the number of monocytes in the blood stream. The effect of actual degeneration of the liver on the monocytes was studied by Holt,[4] who ligated one of the hepatic ducts and found that this operation was followed by a rise in the monocyte count.

In these three conditions, catarrhal jaundice, tetrachlorethane poisoning and ligation of a bile duct, a rise in the monocyte count and injury to the liver were always present. Since the increase in the number of the monocytes occurred with injury to the liver, it seems possible that the increase is a direct result of the injury. It is obvious that, if this hypothesis is correct, the mechanism involved may be one of several. The injury to the liver may bring about a change in its physiologic activities,

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* From the Departments of Anatomy and Medicine, Vanderbilt University School of Medicine.

* Edgar Jones' participation in this work was made possible by a grant from the Henry Strong Denison Medical Foundation.

1. Jones, C. M., and Minot, G. R.: Infectious (Catarrhal) Jaundice: An Attempt to Establish a Clinical Entity; Observation on the Excretion and Retention of the Bile Pigments, and on the Blood, Boston M. & S. J. **189**:531, 1923.

2. Thewlis, Ethel; and Middleton, W. S.: The Leukocytic Picture in Catarrhal Jaundice (Cholangitis), Am. J. M. Sc. **169**:59, 1925.

3. Minot, G. R., and Smith, L. W.: The Blood in Tetrachlorethane Poisoning, Arch. Int. Med. **28**:687 (Dec.) 1921.

4. Holt, R. B.: Effect on White Blood Cells in Rabbit by Ligation of Common Bile Duct, Proc. Soc. Exper. Biol. & Med. **24**:974, 1927.

which, in turn, affects the balance in the production of blood cells. Again, as the result of the degeneration, substances may be set free in the liver which affect the production of monocytes. For example, bilirubin, bile salts, cholesterol, unsaturated fats or other substances may be set free under these conditions and may produce monocytosis by their effect on tissues elsewhere. In view of these considerations, it seemed worth while to investigate the monocyte by studying some of the possible relationships of this cell to liver tissue. It was decided, as the first step in such an investigation, to inject fresh normal liver into animals in order to determine whether this simple procedure would also cause a rise in the monocyte count. Accordingly, a suspension of fresh rabbit liver was injected into a series of rabbits and the effect on the blood noted.

EXPERIMENTAL PROCEDURES

This series included eleven rabbits. These may be divided into three groups. Group 1 includes five animals (nos. 16, 17, 18, 19 and 21) all of which were carefully examined at autopsy for the presence of infections, and only one of which (no. 17) showed any infectious process. This animal had questionable small pneumonic patches in the lungs. All these animals received injections of whole liver. Group 2 includes three animals (nos. 2, 5 and 11). Rabbits 11 and 5 received injections of whole liver. Rabbit 2 received an injection, first, of whole liver and then of perfused liver. None of the animals in this group was controlled by postmortem examination. Group 3 includes three animals (nos. 8, 9 and 15), in all of which peritonitis was disclosed at autopsy. Two of these animals (nos. 9 and 15) received injections of perfused liver.

The preparations of the whole liver were made as follows. A rabbit was killed with ether anesthesia and the liver immediately removed under aseptic conditions. This was either run through a meat chopper or macerated with mortar and pestle, and a saline suspension of it was made. This suspension was strained through gauze and was then injected intraperitoneally. About one half of the suspension from one liver was injected into a single animal, the total volume being approximately from 25 to 30 cc.

The preparations of perfused liver were made as follows. The animal was killed by ether anesthesia and the abdominal cavity opened. A glass cannula was inserted into the hepatic artery, and a large volume of warm sterile physiologic sodium chloride solution was run through the liver. When all the blood had been washed out, the organ was removed and macerated and the suspension made as described.

Total and differential counts were made on all the animals for a period prior to the injection in order that the normal values of these counts might be established. Following the injection, total and differential counts were made daily on all the animals for a period of approximately five days. On five animals (nos. 16, 17, 18, 19 and 21), two counts were made daily for the first two days following the injection. In making the differential counts, the supravital technic was used, with neutral red as the stain.

In addition to the three groups of rabbits mentioned, two other groups of three each were studied. The animals of one of these groups received injections of autoclaved calf liver, while those in the other series received injections of a suspension of calf liver prepared as described, but not heated or otherwise sterilized,

although every possible precaution was taken to prevent the liver's being contaminated. Peritonitis developed in all these animals.

RESULTS

In every instance, the animals in group 1 showed a definite rise in the number of monocytes in the circulating blood in from twenty-four to forty-eight hours after the injection of whole liver into the peritoneal cavity. In three of the animals this rise was maintained for six days, and in the other two for three days. Rabbit 16 showed the highest peak for the monocyte counts thirty-six hours after the injection of liver intraperitoneally. The absolute number of monocytes at this time was 3,257,

Average Blood Cell Counts of Rabbits Before and After Intraperitoneal Injection of Liver

	Neutrophils			Lymphocytes			Monocytes			
Rabbit No.	Before	After	Average Difference in per Cent	Before	After	Average Difference in per Cent	Before	After	Average Difference in per Cent	Remarks
16	7,640	6,487	.....	4,781	2,662	.....	1,168	1,540	.....	Group 1. All of this series were free from infections, except rabbit 17, which showed questionable small pneumonic patches
17	4,776	5,864	.....	2,039	2,053	.....	836	1,379	.....	
18	4,614	4,333	.....	2,907	2,184	.....	1,029	1,024	.....	
19	4,029	5,982	.....	2,023	1,939	.....	786	1,072	.....	
21	4,404	6,449	.....	1,866	1,490	.....	439	1,288	.....	
Aver.	5,093	5,823	+14.3	2,723	2,067	−24.1	852	1,261	+48.0	
11	9,443	3,068	.....	3,073	2,520	.....	648	921	.....	Group 2. This series was not controlled by autopsies; rabbit 2 received an injection first of whole liver and later of perfused liver; A indicates after whole liver; B, after perfused liver
5	4,984	3,387	.....	2,446	2,300	.....	443	780	.....	
2	4,193	A5,591	.....	3,764	A2,620	.....	654	A1,209	.....	
		B5,034			B1,636			B1,419		
Aver.	6,207	A4,015	−35.3	3,094	A2,480	−19.8	582	A 970	+66.7	
		B3,839	−38.3		B2,152	−30.4		B1,040	+78.7	
8	4,395	4,135	.....	2,840	2,260	.....	218	1,392	.....	Group 3. Peritonitis was present in all of this series
9	5,965	2,126	.....	2,401	1,188	.....	1,140	971	.....	
15	4,085	4,721	.....	3,555	2,843	.....	689	1,415	.....	
Aver.	4,815	3,661	−24.0	2,932	2,097	−28.5	682	1,259	+84.6	

whereas the average value before injection was 1,168. There was an associated slight increase in the total number of white blood cells, the count being 13,560. The lowest maximal value for monocytes in this group was 1,810, in rabbit 19. The average monocyte count prior to the injection of liver in this animal was 786, and the total number of white blood cells was 13,400.

There was a general tendency of the lymphocytes in this group to decrease in number following the injection, the average value after injection being 24.1 per cent below the average value before injection. The neutrophils showed a slight increase in numbers following the injection of liver.

In group 2, the same type of reaction as regards the monocytes following the injection of liver was observed, the average increase being

66.7 per cent, which was slightly greater than that in group 1. Rabbit 7 received, first, an injection of whole liver and later one of perfused liver. Following the injection of whole liver, there was a marked temporary rise in the monocyte count to 5,140 with a total white blood cell count of 11,680. The average number of the monocytes during the period of seven days between the injections of whole and of perfused liver was 970. Following the injection of the perfused liver there was no great increase in the number of monocytes, but the curve had a slightly higher level, the average being 1,040. No real difference in the response of the lymphocytes in this group from that in group 1 was observed. The neutrophils, however, showed a definite decrease in number, but this was due to a marked decrease in the number of these cells in rabbit 11. In the other two animals of this group, the curve was somewhat higher after, than it was before, the injection.

In group 3, the animals all had peritonitis, and the only change in response to the injection of liver, as compared with the response in group 1, was a greater increase in the percentage of monocytes (84.6 per cent). However, marked peaks did not occur, and the average absolute value of the monocytes was not high (1,259). It was practically the same as that for group 1 (1,261).

COMMENT

The one outstanding result in this series of experiments was the definite increase in the number of monocytes following the injection of a suspension of liver into the peritoneal cavity. This was a constant observation, and it occurred within from twenty-four to forty-eight hours after the injection. The explanation of this increase is not obvious. Several possibilities present themselves. The simplest explanation is that the increase was due to the degeneration of the liver tissue in the peritoneal cavity with the liberation of some stimulant active in the production of monocytes. Again, it might have been due to the action of the liver, or substances formed from it by chemical changes, on the peritoneum or other tissues locally—i.e., it might have been a kind of foreign body reaction. The third possibility is that the degenerated liver produced substances which, in turn, acted on the liver cells of the animal and brought about the condition that stimulated the production of monocytes.

Of interest is the early marked decrease of the number of monocytes and lymphocytes, with an inconstant reaction of the neutrophils. The monocytes remained decreased in number only for a short period and then increased, whereas the lymphocytes showed an average decrease of 24.1 per cent throughout the period of observation following the injection of liver. So far as this series of experiments goes, it seems

that the factor producing monocytosis was capable of causing a diminution in the number of lymphocytes. Is it possible that the two types of cells are mutually inhibitory?

The changes in the animals with frank peritonitis were different from those in the animals of group 1 in two respects. First, the neutrophils were decreased in number, rather than increased, their average total number after the injection being 24 per cent below their average before injection. Second, the average increase in the percentage of the monocytes was distinctly higher than it was in the group of noninfected animals. May we assume that the number of the monocytes was increased as a result of this infection alone, or was this increase the result of infection in a "sensitized" animal? In other words, is it possible that the number of the monocytes was elevated as a result of some change in the animal following the injection of liver whereby it was more able to produce monocytes in the presence of infection than normally? The decrease in the number of neutrophils is not the ordinary response to purulent infection, but it may be that this was due to massive infection. If so, one would expect the number of monocytes also to be decreased. The same type of reaction occurred when the suspension of calf liver was injected into the animals, in which again peritonitis was present. Autoclaved calf liver, when injected intraperitoneally, did not produce any change of note in the blood counts.

CONCLUSIONS

Rabbits into which rabbit liver has been injected intraperitoneally show in the blood, first, an initial decrease in the number of lymphocytes and of monocytes and later a decided increase in the number of monocytes with continued diminution in the number of lymphocytes. The number of neutrophils is slightly increased.

FATTY CHANGES IN THE KUPFFER CELLS IN THE LIVER OF THE GUINEA-PIG IN PHOSPHORUS POISONING*

JOHN S. LAWRENCE, M.D.
AND
M. M. HUFFMAN, M.D.
NASHVILLE, TENN.

In the course of an investigation on the effect of phosphorus on the white cells of the blood of the guinea-pig, routine histologic examination of the liver revealed an increased amount of fat.[1] This fat seemed to be largely in the Kupffer cells and this uniformity of distribution suggested the extension of these experiments to a larger series of animals.

Eighty guinea-pigs were studied in this connection. Forty of these animals were controls and forty received phosphorus, either subcutaneously or by mouth. Of the forty animals that received phosphorus, twenty-eight were given subcutaneous injections of from 0.5 to 1 mg. of yellow phosphorus in oil, repeated at intervals of from three to five days, until each animal had received from 1.5 to 5.5 mg. in all. The other twelve animals were each given repeated doses of from 1 to 2 mg. of phosphorus by mouth, for from six to thirteen days.

The interval of time elapsing between the administration of phosphorus to an animal and the postmortem examination varied widely, the shortest interval being two days and the longest thirty-nine days. Some of the animals died, but the great majority were put to death. Specimens of the livers were fixed in a diluted solution of formaldehyde U. S. P. (1:10), and frozen sections were cut and stained in hematoxylin and scharlach r.

RESULTS

Fourteen, or one half, of the animals receiving phosphorus subcutaneously had an accumulation of fat in the liver. Twelve of the animals showing fatty changes had a definite arrangement of the fat. In these animals, the fat showed a decided preference for the Kupffer cells, thus giving the section the appearance of a network of fat. Four of the guinea-pigs that had fat in the Kupffer cells also showed some fat in the

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* From the Departments of Medicine and Anatomy, Vanderbilt University School of Medicine.

1. Huffman, M. M.; Lawrence, J. S., and Jones, Edgar: The Effect on the White Blood Cells Produced by the Intraperitoneal Injection of Whole Liver, Arch. Path., this issue, p. 804.

hepatic cells. In these four, the periportal areas showed an accumulation of fat in all the Kupffer cells and in some of the hepatic cells. As one approached the center of the lobule, an area was found in which only the Kupffer cells showed fatty globules, and finally, on reaching the cells in the immediate neighborhood of the central vein, fat was not found. There were, then, three distinct zones as regards the accumulation of fat, viz., a periportal zone with fat in both the hepatic and the Kupffer cells, a middle zone with involvement of the Kupffer cells alone, and a central zone without fat in any of the cells. The globules of fat tended to be of moderate size, the average size being considerably less than that of the nuclei. The other two animals that showed positive signs of changes in the liver did not present this arrangement. In one there was a diffuse involvement with fat, which was present in considerable amounts. However, the central areas, in general, seemed to be spared. The fat globules varied in size from some with a diameter equal to that of the nuclei to others like small particles. In the other animal there was only a small excess of fat. This was not located particularly in the Kupffer cells, but chiefly in the liver cells. A preference was not shown for either the periportal or the central areas. The globules of fat, on the average, were of the size of the nuclei. The epithelium of one of the bile capillaries showed a small amount of fat. The other fourteen animals into which subcutaneous injections of phosphorus had been made, did not show any evidence of an increase of fat in the liver by the technic used, viz., frozen sections of tissue fixed in a diluted solution of formaldehyde, U. S. P. (1:10) and stained with scharlach r.

Of the twelve animals given phosphorus by mouth, only two failed to show an accumulation of fat in the liver. Of those showing fatty changes, four showed a decided tendency for the fatty globules to appear in the Kupffer cells; six did not show this arrangement of the fat. All these animals had an extensive accumulation of fat, both the hepatic and the Kupffer cells containing an excess of the fatty globules. The general tendency was for the central area to be spared, three animals definitely showing this arrangement of the fat. Two of the animals treated with phosphorus by mouth did not show fatty changes in the liver.

There was a general tendency throughout the group of animals treated with phosphorus, and showing fatty changes, for the central areas to be spared at the expense of the periportal areas. The epithelium of the bile capillaries contained a remarkably small amount of fat. In only five of sixteen animals (approximately one third) particularly studied with reference to this point was there any accumulation of fat in the biliary epithelial cells, and in these there was only a small amount.

COMMENT

The great predilection of the fatty globules for the Kupffer cells is worthy of note. Twelve of fourteen animals showing fatty changes in the liver as a result of phosphorus poisoning presented this predilection. The occurrence was so regular that it does not seem that it can be questioned. It may be noted that a much higher percentage of the animals given phosphorus by mouth showed fatty changes of a more generalized nature. Eleven of the animals showing this generalized fatty degeneration had an extensive degenerative process in the liver. In consideration of the data presented—the typical and regular arrangement of the fat in the Kupffer cells, the lesser degree of fatty involvement in these animals, the wavelike arrangement of the fat in four guinea-pigs and, finally, the generalized involvement, with implication of both the Kupffer and the hepatic cells, but with a tendency to spare the central areas—it seems plausible to assume that one of the earliest changes in phosphorus poisoning is an accumulation of fatty globules in the Kupffer cells of the periportal areas. The next step, as regards the fat, is probably an involvement of Kupffer cells in the middle zones and the hepatic cells in the periportal areas. Later, the Kupffer cells in the central area are involved and the hepatic cells of the middle zones. Finally, all the zones and all the cells are involved. In other words, the process advances from periphery to center and from Kupffer cell to hepatic cell.

Questions arise as to the significance of the fat in the Kupffer cells. 1. Does the fat accumulate in these cells as the result of a lipemia and the secondary phagocytosis of the fatty droplets? 2. Is the appearance of fat merely a matter of changes in its character such that it can be shown by staining? In other words, is there no increase in the actual amount of fatty material in the cells but a change in the character of the fat, so that it stains with scharlach r, whereas, before this change, it did not stain in this way. 3. Is the fat that appears in the Kupffer cells the result of degeneration of the cytoplasm itself wherein fat is formed by changes of a chemical character involving the destruction of the cell cytoplasm? Our evidence does not thus far permit any answer to these questions. In this connection, it is of interest to consider the possible relationship between the involvement of the Kupffer cells and the increase in the number of monocytes in the circulating blood in animals treated with phosphorus subcutaneously. That the increase in monocytes is not always dependent on fatty infiltration of the Kupffer cells has been shown by Lawrence and Huffman.[2] However, fatty changes in the Kupffer cells may have been present in some

2. Lawrence, J. S., and Huffman, M. M.: An Increase in the Number of Monocytes in the Blood Following Subcutaneous Administration of Yellow Phosphorus in Oil, Arch. Path., this issue, p. 813.

of the animals showing increased numbers of monocytes in the blood at the time of the increase, and these may have disappeared later, since, as has been known for a long while, animals show fatty changes in the liver early in phosphorus poisoning, which may later disappear.[3]

The large volume of recent work concerning the question of the relationship between the monocyte and the clasmatocyte renders it necessary to raise the question whether the changes in the Kupffer cells that took place following the administration of phosphorus could be a direct cause of the monocytosis occurring in these animals.[2] The evidence in our series of experiments argues against any such direct connection, since none of the monocytes in the circulating blood contained fat and none of them, furthermore, were in any way similar to the Kupffer cell as examined free from the liver on fresh supravital smears. On the other hand, our results do not permit definite statement regarding the question of the relationship between these two types of cells. This question has been thoroughly discussed elsewhere.[4]

The failure of sixteen of the forty animals to show any fatty changes in the liver should be noted. In these animals, the average number of days elapsing after the administration of phosphorus was 15.3 against 8.8 days for those showing fatty changes. The average total dosage of phosphorus for these animals was 2 mg., while that for the animals showing a typical accumulation of fat in the Kupffer cells was 2.1 mg. These facts seem to indicate that the animals in this group were less susceptible to phosphorus than the others. However, the explanation of the difference in reaction may be found in the failure of absorption of the material from the subcutaneous tissue in certain of the animals. Normally, the liver of the guinea-pig contains only a small amount of fat. In our group of forty control animals, the liver in only one case showed more than the faintest demonstrable trace of fat.

CONCLUSION

Administration of phosphorus in small amounts to guinea-pigs produces an accumulation of fatty globules in the Kupffer cells as an early manifestation of phosphorus poisoning of the liver.

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AN INCREASE IN THE NUMBER OF MONOCYTES IN THE BLOOD FOLLOWING SUBCUTANEOUS ADMINISTRATION OF YELLOW PHOSPHORUS IN OIL *

JOHN S. LAWRENCE, M.D.
AND
M. M. HUFFMAN, M.D.
NASHVILLE, TENN.

In recent years, a fairly large number of observations, both clinical and experimental, have been reported which indicate that there may be some obscure relationship between injury to the liver and the number of the circulating monocytes. The clinical observations include the changes in catarrhal jaundice [1] and those in accidental poisoning by tetrachlorethane.[2] The experimental observations that indicated this relationship of liver and monocyte were those of Holt [3] and of Huffman, Lawrence and Jones.[4] Holt observed that the ligation of a bile duct in the rabbit produced a marked and relatively well sustained increase in the number of monocytes; while Huffman, Lawrence and Jones injected sterile, macerated liver from normal rabbits into rabbits intraperitoneally and found a marked rise in the number of monocytes following this procedure.

In all these conditions, the one factor in common was the presence of injured liver tissue—if one can assume that in catarrhal jaundice the liver is actually injured. In consideration of this fact, namely, that the number of the monocytes in the circulating blood is increased in several conditions in which there is present injured hepatic tissue, the importance of further study of degeneration of the liver seemed indicated. In considering what type of degeneration of the liver to study, it seemed wise

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* From the Departments of Medicine and Anatomy, Vanderbilt University School of Medicine.

1. Jones, C. M., and Minot, G. R.: Infectious (Catarrhal) Jaundice: An Attempt to Establish a Clinical Entity; Observations on the Excretion and Retention of the Bile Pigments, and on the Blood, Boston M. & S. J. **189**:531, 1923. Thewlis, Ethel, and Middleton, W. S.: The Leucocytic Picture in Catarrhal Jaundice (Cholangitis), Am. J. M. Sc. **169**:59, 1925.

2. Minot, G. R., and Smith, L. W.: The Blood in Tetrachlorethane Poisoning, Arch. Int. Med. **28**:687 (Dec.) 1921.

3. Holt, R. B.: Effect on White Blood Cells in Rabbit of Ligation of Common Bile Duct, Proc. Soc. Exper. Biol. & Med. **24**:974, 1927.

4. Huffman, M. M.; Lawrence, J. S., and Jones, Edgar: The Effect on the White Blood Cells Produced by Intraperitoneal Injection of Whole Liver, Arch. Path., this issue, p. 804

to begin with that produced by the use of yellow phosphorus—the reason being that the degeneration produced by phosphorus is gradual and is characterized especially by a periportal distribution of the earliest changes. The literature bearing on this point is discussed elsewhere.[5]

EXPERIMENTAL METHOD

This report is based on the results obtained in nineteen guinea-pigs. Thirteen of these animals received subcutaneous injections of phosphorus, and six were given phosphorus by mouth. Those receiving phosphorus subcutaneously received the injections at intervals of from three to five days, the individual dosage in the great majority of the cases being 0.5 mg. Five of these animals received a few injections of 1 mg. each. The smallest total dosage was 3.5 mg., the largest 5.5 mg. of phosphorus. The preparation used was made by Hynson, Westcott and Dunning from a stock solution of yellow phosphorus in almond oil. This stock solution contained 1 per cent of yellow phosphorus. Ten cubic centimeters of this solution was mixed with 90 cc. of olive oil, thus making 1 cc. of the final mixture equivalent to 1 mg. of yellow phosphorus.

Total and differential counts of the white cells were made daily for several days preceding and throughout the period of the injections of phosphorus. The supravital method was used in making all the differential counts.

Three of the six animals given phosphorus by mouth were given daily dosages of 1 mg. for from six to seven days; one was given 2 mg. daily for six days, and the remaining two were given 1 mg. every other day for six days, and then, after an interval of three days, a dose of 1 mg.

Complete autopsies were made on all the animals of both series, except guinea-pigs 38, 39, 40 and 41, which were used for further investigation and were in good condition at the time that the blood studies were discontinued.

An examination of the liver for fat (by staining frozen sections with scharlach r) was made in each case. Guinea-pigs 13, 14, 19, 22, 27, 28, 29, 30 and 31 showed evidence of phosphorus poisoning in the liver. Guinea-pigs 11, 18 and 24 showed questionable signs of phosphorus poisoning in the liver. Guinea-pigs 12, 24 and 26 did not reveal any fatty changes in the liver. Guinea-pig 13 had pneumonia. There was questionable pneumonia in guinea-pig 14. Guinea-pig 22 showed hemorrhage in the suprarenal glands. Guinea-pigs 13, 14, 22 and 27 died. The rest of the animals were killed.

In brief, these experiments resulted in the observation that in the early stages of phosphorus poisoning there is a marked increase in the number of monocytes in the circulating blood.

RESULTS

The animals that received subcutaneous injections of yellow phosphorus are divided into three groups, according to whether they showed a definite response in the blood, a questionable or equivocal response or no response following the injection of phosphorus. Group 1 consists of six guinea-pigs all of which showed definite changes in the number of circulating white cells following the injection of phosphorus. In each instance, the striking observation was an appreciable rise in the number

5. Lawrence, J. S., and Huffman, M. M.: Fatty Changes in the Kupffer Cells of the Liver of the Guinea-Pig in Phosphorus Poisoning, Arch. Path., this issue, p. 809.

of monocytes. The highest value found was in guinea-pig 11 after the third injection of phosphorus. The absolute number of monocytes at this time was 4,544, which was an increase of 4,002, or 738.4 per cent, over the average value of 542 found before the injection. The lowest maximal monocyte value for any animal in this series was 2,225, in

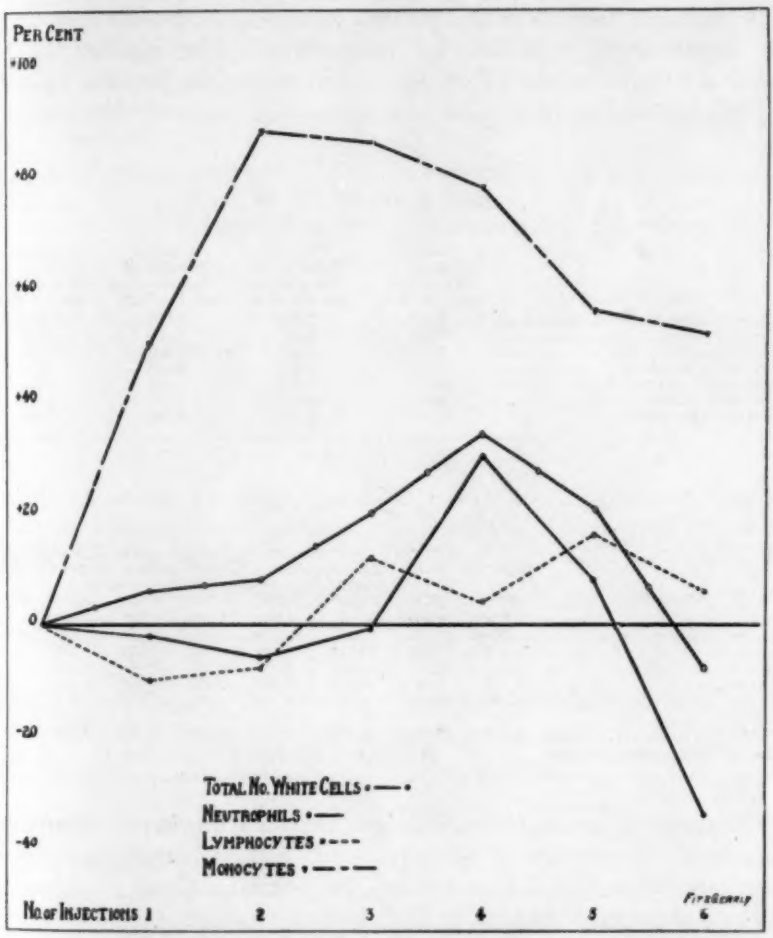

Chart 1.—Average percentage variations in the blood counts of animals in group 1, following repeated injections of phosphorus.

guinea-pig 41, after the fourth injection of phosphorus. This was an increase of 1,332, or 149.2 per cent, over the value of 893 found before the injection.

This monocytic response was greatest after the second and the third injections of phosphorus (chart 1). The maximal average percentage increase (87.9 per cent) in this experiment occurred after the second

injection. Changes of note did not occur in the total number of white cells, or in the absolute number of neutrophils or lymphocytes. The low peak reached by the curve of the total white cell counts and the curve of the neutrophil counts occurred considerably after the peak had been reached by the monocytes. Table 1 represents the average values in a typical animal (guinea-pig 40).

Group 2 includes three animals that showed an equivalent response to the subcutaneous injections of phosphorus. The number of the monocytes tended to run a level course, and in only one instance (guinea-pig 25) did the number show any appreciable increase. In this case

TABLE 1.—*Effect of Injection of Yellow Phosphorus on the Blood Counts in Guinea-Pig 40*

Time of Count	Average Number of White Cells per Cu. Mm.	Average Number of Neutrophils per Cu. Mm.	Average Number of Lymphocytes per Cu. Mm.	Average Number of Monocytes per Cu. Mm.
Before injections of phosphorus....	8,640	7,008	2,320	971
After first injection.....	7,563	3,636	1,980	1,535
After second injection.....	9,017	4,680	1,909	1,740
After third injection.....	9,050	4,252	2,198	1,720
After fourth injection.....	9,725	4,512	2,549	1,818
After fifth injection.....	9,275	4,727	2,162	1,580
After sixth injection.....	7,250	2,791	2,249	1,486

TABLE 2.—*Average Blood Counts in Animals Given Phosphorus by Mouth*

	Total per Cu.Mm.		Neutrophils per Cu.Mm.		Lymphocytes per Cu.Mm.		Monocytes per Cu.Mm.	
Guinea-Pig	Before	After	Before	After	Before	After	Before	After
22.....	6,784	8,000	5,110	4,024	1,539	2,523	868	876
27.....	8,750	15,843	4,990	9,876	2,555	3,437	1,037	2,206
28.....	18,400	17,533	10,872	11,888	3,191	3,534	2,252	2,034
29.....	14,675	10,900	4,861	6,017	6,981	2,960	1,882	1,671
30.....	6,950	9,083	2,619	3,892	3,315	3,931	705	825
31.....	10,583	10,850	3,938	5,718	4,620	3,227	811	1,003
Average.....	11,024	12,035	5,400	6,903	3,700	3,219	1,259	1,461
Average difference in per cent		+9.2		+27.8		−13.0		+16.0

the increase was associated with an increase in the number of neutrophils. In general, the number of neutrophils and that of lymphocytes did not show any appreciable change from the normal. Chart 2 shows the average percentage changes in the blood counts of the animals in this group following injections of phosphorus.

Group 3 consists of four animals that did not show any changes in the curves of the white cell counts. These animals were treated in the same way as those of groups 1 and 2, but they failed to respond.

The six animals given phosphorus by mouth did not exhibit the same response as those given phosphorus subcutaneously. Four of these animals did not show any variations in the blood counts beyond normal limits. The remaining two animals showed some changes in the curves. One of these animals, guinea-pig 27, showed a marked terminal increase

in the number of monocytes associated with a rather marked leukocytosis. The maximal number of monocytes in this case was 5,615, and the total number of white cells at this time was 31,200. The second animal showing an increase in monocytes was no. 31. The maximal value for monocytes in this animal was 2,184, but the average value after the

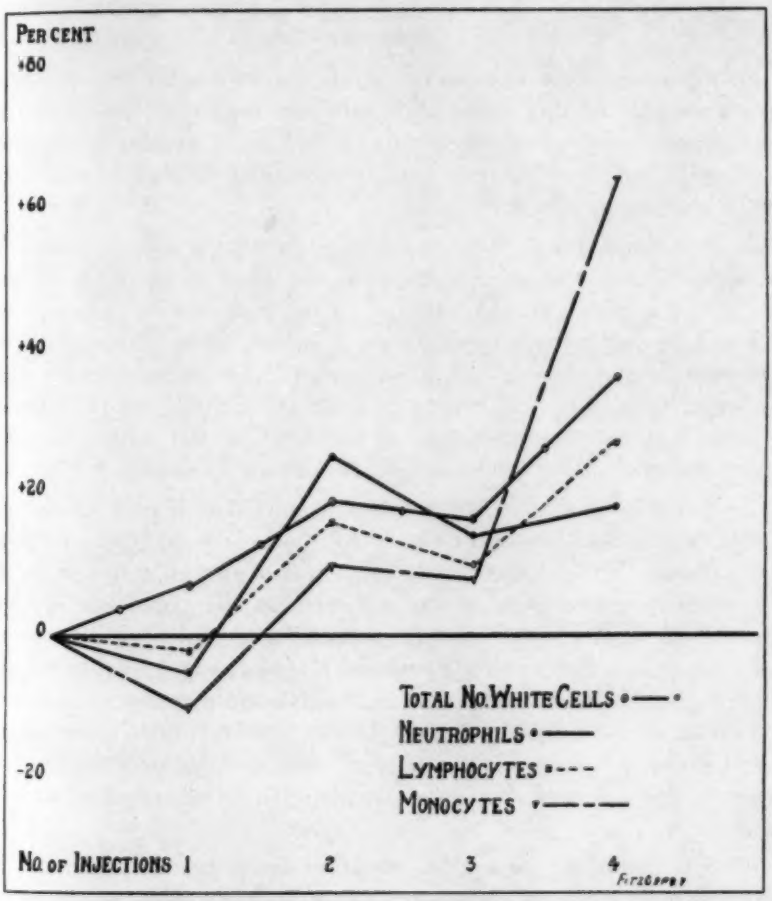

Chart 2.—Average percentage variations in the blood counts of animals in group 2 following repeated injections of phosphorus.

injection of phosphorus was only 1,063. The other white cells did not show any variation beyond the normal in this group of animals.

In addition to the aforementioned animals treated with phosphorus, two guinea-pigs (nos. 124 and 125) were treated with repeated subcutaneous injections of 0.5 cc. of olive oil and two (nos. 126 and 127) with repeated subcutaneous injections of 0.5 cc. of almond oil. The two guinea-pigs given olive oil showed absolutely no increase in

the number of monocytes in the blood stream. Those given almond oil did not present so level a monocytic curve, but the variation was within the normal limits. The highest value for monocytes in either of these animals was 1,627 after the injections against 1,118 prior to the injections. This high value was present on only one occasion, the curve soon returning to a value below 1,000 per cubic millimeter.

COMMENT

An increase in the number of circulating monocytes was found in the guinea-pigs of this series with sufficient regularity, following the subcutaneous injection of phosphorus, to indicate a definite relationship between the monocytic increase and the injections of phosphorus. This suggests several possibilities.

1. Was the increase in the number of circulating monocytes due to the phosphorus per se, or was it due to the olive oil or to the almond oil? That almond oil and olive oil alone were not responsible was indicated by the control experiments in which these substances were used without phosphorus. In these control experiments, changes were not found in the white cell counts. On the other hand, that phosphorus, as such, was not the only factor is indicated by the failure to get a similar response in the animals given phosphorus by mouth.

2. Were the variations in the blood counts due directly to changes in the liver? Such a conclusion is not borne out by the pathologic observations. Table 1 shows that there was a marked response of the monocytes in guinea-pigs 11 and 12, whereas the pathologic changes in these two animals did not give conclusive evidence of phosphorus poisoning in the liver, the observations in guinea-pig 12 being entirely negative in this regard. The failure to demonstrate changes in the liver by examination of sections stained for fat, however, does not eliminate the possibility of changes being present. It is possible that variations in structure and function that cannot be detected by this method may be present.

3. Can the blood picture be explained by a general effect on the animal organism of some substance or substances produced by the action of phosphorus on the tissues? This seems a possibility, but we did not have any direct evidence in its favor.

4. Are the monocytes in the blood stream derived from the tissues at the site of the injection, and, if so, is there a sufficient reaction quantitatively to account for the number of monocytes in the circulating blood? Sufficient evidence to answer this definitely has not yet been accumulated, as is indicated in the discussion on the tissues published elsewhere.[6]

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6. Lawrence, J. S.; Tompkins, Edna H., and Cunningham, R. S.: The Production of Monocytes and Epithelioid Cells in Subcutaneous Tissue by Injection of Various Irritants, Proc. Soc. Exper. Biol. & Med. **26**:331, 1929.

The effect of pneumonia on the blood counts of guinea-pig 13 is of interest; the greatest average increase in the number of monocytes in this animal occurred after the first injection of phosphorus and at a period when pneumonia was probably not present. On the other hand, the question of pneumonia cannot be entirely discarded, for it has been shown that there is an increase in the number of monocytes during the period of resolution.[7]

An analysis of the variations in the total number of white cells and in the absolute number of neutrophils and lymphocytes fails to reveal any indications of abnormality. This points definitely to an effect confined entirely to the monocytes.

The increase in the number of monocytes is as constant and as great as in tuberculosis. The question naturally arises whether there is, etiologically, any relationship between the monocytosis of tuberculosis and that following the administration of phosphorus. Of course, it is well known that there is an appreciable amount of phosphorus in the tubercle bacillus. And recently Sabin and Doan[8] showed that the phosphatide fraction of the tubercle bacillus is a potent stimulus to the production of monocytes. Obviously, it is possible that the two reactions have both phosphorus and fat in common, but whether the relationship can be carried any further is not apparent at present.

CONCLUSION

Subcutaneous injections of yellow phosphorus cause a marked increase in the number of the circulating monocytes.

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7. Hickling, R. A.: The Monocytes in Pneumonia: A Clinical and Hematologic Study, Arch. Int. Med. **40**:594 (Nov.) 1927.

8. Sabin, Florence R., and Doan, C. A.: The Biological Reactions in Rabbits to the Protein and Phosphatide Fractions from the Chemical Analysis of Human Tubercle Bacilli, J. Exper. Med. **46**:645, 1927.

THE ORIGIN AND FATE OF SICKLE-SHAPED RED BLOOD CELLS *

JOSEPH LEVY, M.D.
NEW ROCHELLE, N. Y.

Sickle-shaped red blood corpuscles are found so constantly in certain chronic anemias,[1] in unexplained fevers [2] and in sickle cell anemia,[3] and the prevailing views as to their method of origin and ultimate fate are so conflicting that it is important to elucidate the mechanism by which these cells assume their abnormal shape.

Though Hayem [1] had long since drawn attention to these peculiarly shaped cells, it remained for the Sergent brothers [2] to describe them in detail as the "corps en demi-lune." Furthermore, they demonstrated [4] that these cells are not artefacts, as was maintained by Hayem and by Nicolle and Comte.[5] Laveran,[2] in the discussion of the observations of the Sergents, expressed the view that the cellular defect is due to the departure of a parasite. Others [6] maintained that the cells are produced by an altered osmotic condition of the cell membrane. Still another conception is that the surface tension of the cell and serum is altered or that the phenomenon of adsorption occurs.[7] Many believe that it is merely a mechanical distortion.[8] Langeron [9] produced sickle cells in animals by injecting them with massive doses of lead. Hahn

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* From the New Rochelle Hospital.

1. Hayem, Georges: Du sang et de ses altérations anatomiques, 1889, Paris, G. Masson (Masson et Cie.), p. 336.

2. Sergent, E., and Sergent, É.: Sur des corps particuliers du sang des paludéens, Compt rend. Soc. de biol. **58**:51, 1905.

3. Herrick, J. B.: Peculiar Elongated and Sickle Shaped Red Blood Corpuscles in a Case of Severe Anemia, Arch. Int. Med. **6**:517 (Nov.) 1910.

4. Sergent, E., and Sergent, É.: Sur les corps en anneau et en demi-lune du sang des paludéens, Compt. rend. Soc. de biol. **59**:252, 1905; Études épidemiologiques et prophylactiques du paludisme, Ann. de l'inst. Pasteur **20**:245, 1906.

5. Nicolle, C., and Comte, C.: Sur la signification des corps en anneau décrits par MM. Sergent dans le sang des paludéens, Compt. rend. Soc. de biol. **58**:760, 1905.

6. Leede, W. N.: Gigantozyten bei Malaria, Mitt. a. d. Hamburg. Staatskrankenanstalten **13**:1, 1912.

7. Josephs, H. W.: Sickle Cell Anemia, Bull. Johns Hopkins Hosp. **40**:77, 1927.

8. Brumpt, E.: Globules géants ou "corps en demi-lune" du paludisme, Bull. Soc. path. exot. **1**:201, 1908.

9. Langeron, M.: Hématies en demi-lune dans le sang du rat et du cobaye, Compt. rend. Soc. de biol. **70**:434, 1911.

and his co-workers [10] thought that asphyxia and anoxemia are causal factors. Many have named the spleen as the seat of the trouble.[11] Cooley and Lee [12] thought that the red blood cells possess a peculiar vulnerability to some hemolytic agent. Emmel [13] suggested that the manner in which these blood cells are transformed may be due to an abnormal activity of the same factors that cause the original erythrocyte to change to the biconcave disk.[14] That the anomaly is a congenital [15] or an anatomic defect [16] is supported by the reports of its occurrence in families.[17]

The variety of opinions as to the origin of sickle cells is due to the difficulty in seeing their change from the normal to the abnormal shape.[18] While it may be possible to establish certain criteria for the change by experimental modification, it seems that the mechanism of the alteration can be ascertained only by a detailed microscopic study of single cells.

MATERIAL AND METHODS

During the past year, fresh blood smears were made as a matter of routine for all negroes on admission to the hospital. A minute drop of the patient's blood was obtained on a cover slip. This was inverted over a glass slide. The rim of the cover slip was sealed with petrolatum or Canada balsam. Only smears that showed red blood cells singly, just touching, but not overlapping, or in rouleaux, were accepted for further study. Such smears can be secured by having the glassware scrupulously clean, the patient's finger free from grease and the size of the drop of blood just large enough to give a very thin spread. Though there were many failures, results were usually obtained by using new glassware, copious quantities of ether and alcohol and infinite patience. There were 213 colored patients in the series, twelve of whom showed sickle-shaped blood cells in peripheral and venous blood. Smears from these twelve cases were further studied by placing

10. Hahn, E. V., and Gillespie, E. B.: Sickle Cell Anemia: Report of a Case Greatly Improved by Splenectomy; Experimental Study of Sickle Cell Formation, Arch. Int. Med. **39**:233 (Feb.) 1927. Hahn, E. V.: Sickle Cell (Depranocytic) Anemia, Am. J. M. Sc. **175**:206, 1928.

11. Dreyfoos: Sickle Cell Anemia, Arch. Pediat. **43**:436, 1926.

12. Cooley, T. B., and Lee, P.: The Sickle Cell Phenomenon, Am. J. Dis. Child. **32**:334 (Sept.) 1926.

13. Emmel, V. E.: A Study of the Erythrocyte in a Case of Severe Anemia with Elongated and Sickle Shaped Red Blood Corpuscles, Arch. Int. Med. **20**: 586 (Oct.) 1927.

14. Castana, V.: I gigantocite e le anemia semilunari, Pediatria **33**:431, 1925.

15. Dresbach, M.: Elliptical Human Red Corpuscles, Science **19**:469, 1904; **21**:473, 1905.

16. Bishop, F. W.: Elliptical Human Erythrocytes, Arch. Int. Med. **14**:388 (Sept.) 1914.

17. Sydenstricker, V. P.: Further Observations on Sickle Cell Anemia, J. A. M. A. **83**:12 (July 5) 1924.

18. Cook, J. E., and Meyer, J.: Severe Anemia with Remarkable Elongated and Sickle Shaped Red Blood Cells and Chronic Leg Ulcer, Arch. Int. Med. **16**:644 (Oct.) 1915.

the slide beneath the high power and oil immersion objectives of the microscope, fixing the slide in such a manner that it could not be moved and making camera lucida and free hand drawings of the changes going on in selected cells of a particular field every fifteen minutes. Simultaneous studies were conducted with the blood at room temperature, 0 C., and 37.5 C. It was thus possible to ascertain when and how the cells changed their shape, as well as the percentage of cells that remained normal after a given period.

THE ORIGIN OF SICKLE CELLS

That the appearance of sickle-shaped red blood cells is subject to considerable variation has been repeatedly observed.[19] Some of the factors that seem to determine these differences are the clinical condition of the patient, the degree of anemia and the temperature of the

Fig. 1.—Camera lucida and free hand sketches of successive stages in the changing of red cells to sickle shape. *A* represents the cells as they appear immediately after their withdrawal from the blood stream; *B*, one hour later; *C*, two hours later; *D*, four hours later; *E*, twenty-four hours later, and *F*, seventy-two hours later.

blood in vivo and in vitro. While an increase in temperature hastened the phenomenon, a decrease retarded and in many cases actually inhibited it. Occasionally, sickle cells could be found in a smear immediately following the removal of the corpuscle from the blood stream (fig. 1 *A*). Usually, one hour elapsed before the initial changes could be recorded. These consisted in the protrusion of short and long processes from the surface of the cell (fig. 1 *B*). In some cells, the processes assumed the proportions of pseudopodia; in others, they appeared as mere con-

19. Anderson, H. B.: Sickle Cell Anemia, Am. J. M. Sc. **171**:641, 1926.

strictions in the cell membrane. There was a continual protrusion and retraction of these processes. The speed with which this sometimes occurred was startling. Kite [20] observed that a long process in the normal red blood corpuscles of man could be retracted in less than a second. With this continual and rapid rearrangement, it was not surprising to find that in the course of the second hour many of the cells had assumed an abnormal shape. In the majority of instances, especially in the so-called latent cases of sickle cell anemia, the projection of processes with consequent flattening and elongation of the corpuscle resulted in the production of sickle-shaped red blood cells.

In addition, some of these cells actually divided into two unequal parts (fig. 1 *C*). A few of the daughter cells proceeded to subdivide (fig 1 *D*). All the divisions were indirect and the plan of the segmentation varied considerably. Jolly,[21] following the division of the red blood cells in young tritons for fifteen days in vitro, found that pressure determined the plan of segmentation, but did not initiate or inhibit division. On the other hand, Krehl and Marchand [22] quoted the experiences of Schultze, Rollet and Ranvier, who brought about division of red blood cells into microcytes and poikilocytes in vitro by pressure on the cover glass or by gently heating the fresh preparation. In repeating such experiments, it was found that while in preparations of normal blood one could easily cause the disintegration of the red blood cells by pressing on the coverslip, it was not possible to do sc in cases that eventually showed sickle-shaped cells. The cells from these patients showed a rubber-like resilience and, though pressure could distort their shapes considerably, the cells did not disintegrate, and, when the pressure was removed, the cells returned to their characteristic form. Pressure on cells that had already assumed the sickle shape, but which did not show any tendency to divide, also brought out the pliability of these cells; yet again they did not break up or return to their original spherical shape. It seems, therefore, that the phenomenon observed was an actual division of the red blood cell and not a mechanical artefact.

Those corpuscles that become sickle-shaped before or after dividing are in reality embryonic cells. For in the same field as that in which the division or the projection of processes is being observed, one may find certain cells extruding their nuclei (fig. 2 *B*). Following the extrusion of the nucleus, the mother cell, at first cup-shaped, rapidly becomes sickle-shaped. The method by which the change to the sickle

20. Kite, G. L.: Some Structural Transformations of the Blood Cells of Vertebrates, J. Infect. Dis. **15**:319, 1914.

21. Jolly, J.: Recherches expérimentales sur la division indirecte des globules rouges, Arch. d'anat. micr. **6**:455, 1903.

22. Krehl, L., and Marchand, F.: Handbuch der allgemeinen Pathologie, Leipzig, S. Hüzel, 1908, vol. 1, p. 51.

shape occurs is by the extension of pseudopodia in the manner mentioned. Furthermore, as has been shown repeatedly, stained smears in active cases of sickle cell anemia contain large numbers of normoblasts.[23] That immature red blood cells may have the power of independent multiplication was long ago demonstrated by Howell.[24] Phylogenetically, direct amitotic division has been demonstrated in the cold-blooded animals,[25] while Mencl [26] illustrated the division of nucleated red blood cells in *Scorpaena.*

The evidence shows that sickle-shaped red blood cells are formed from embryonic normoblasts that have prematurely entered the general circulation; these cells then continue their maturation by dividing or extruding their nuclei; the resultant cells still immature are then unable to assume the biconcave disk form, but continue as ameboid shapes until an alteration is effected that gives them a favorable nidus.

Fig. 2.—Camera lucida and free hand sketches of successive stages in the transformation of red blood cells into sickle-shaped cells. *A* represents the cells as they appear fifteen minutes after their removal from the blood stream; *B*, one and a half hours after; *C*, one and three quarters after; *D*, two hours after; *E*, three hours after; *F*, twelve hours after, and *G*, nine days after.

THE FATE OF SICKLE CELLS

While it seems plausible that the sickle cells may form as a result of a disturbance in erythropoiesis, it is difficult to understand why some corpuscles assume the form of the biconcave disk (fig. 1 *F*), while

23. Huck, J. G.: Sickle Cell Anemia, Bull. Johns Hopkins Hosp. **23**:335, 1923.

24. Howell, W. H.: The Life History of the Formed Elements of the Blood, Especially the Red Blood Corpuscles, J. Morphol. **4**:57, 1890.

25. Zillberberg, L. A.: On the Direct Amitotic Division of Red Corpuscles of Cold Blooded Animals, Russk. Arch. Patol. Klin. Med. i. Bakteriol. **11**:545, 1901.

26. Mencl, E.: Direkte Teilung von roten Blutkörperchen bei Scorpaena, Anat. Anz. **37**:539, 1910.

others retain their bizarre shape (fig. 2 *G*). Though Oliver [27] demonstrated the separation of spinous and elongated processes from normal erythrocytes in man, such a phenomenon is not found in sickle cells. In a long continued study of such corpuscles in the hermetically sealed wet smears, one is struck not only by the great variations in shape from hour to hour but also by the individual differences between supposedly sister cells. The latter may be accounted for by the angle or plane through which the cell is viewed. Jordan [28] showed that the red blood corpuscle is normally a circular biconcave disk but appears to be cup shape when seen obliquely, and dumb-bell shape when seen in profile.[29] While a few of the cells of abnormal shape were seen to retract their processes immediately and become round, the great majority continued to project new but blunter pseudopodia (fig. 1 *E*). This continued for many hours until finally all the processes were retracted and one found only circular, cup-shaped or peculiarly indented cells (fig. 1 *F*).

Under certain conditions, especially in those patients showing sickle cells without symptoms, the change from the sickle shape to the original form did not occur. After the initial alteration in shape, the cells had not the faculty to change further; they retained their bizarre appearance for an indefinite time (fig. 2 *G*).

The speed of the phenomenon of reversion varied with the temperature at which the blood was kept. The changes depicted in figures 1 and 2 were those that occurred at room temperatures of from 18 to 24 C. When the smears were kept in the incubator at body temperature (37.5 C.), these changes were completed within twenty-four hours. Later changes consisted in the rapid fragmentation of the red blood corpuscles, until one observed numerous microcytes mingled with occasional cells of normal size. Many of the microcytes were phagocytosed by the white corpuscles. Further fragmentation was sometimes prevented by removing the blood from the incubator and placing it in the refrigerator at 0 C. When freshly drawn blood from patients, who normally showed sickle-shaped cells was placed immediately at 0 C. the development of sickle cells rarely occurred. But if the blood was allowed to remain at room temperature for an hour or two and then was placed in the refrigerator, one could demonstrate the presence of sickle-shaped cells in the preparations for as long as three months. In other words, refrigeration acted as a fixative, while incubation at body temperature caused the destruction of the red cells by fragmentation. Rous

27. Oliver, W. W.: The Crenation and Flagellation of Human Erythrocytes, Science **40**:645, 1914.

28. Jordan, H. E.: The Shape of the Human Red Blood Corpuscles, Proc. Soc. Exper. Biol. & Med. **12**:167, 1914.

29. Radasch, H. E.: Observations upon the Form of the Red Blood Corpuscles of Man, Am. J. M. Sc. **131**:837, 1906.

and Robertson [30] found that erythrocytes of normal and anemic rabbits were destroyed by fragmentation.

CONCLUSIONS

The change to the sickle shape observed in red cells is a phase in a profound disturbance of the formation of red blood cells. Embryonic red blood cells and normoblasts enter the systemic circulation. These cells proceed to divide or extrude their nuclei. The resultant cells project pseudopodia and assume bizarre shapes. Heat (body temperature) hastens the change; cold (0 C.) inhibits and may actually prevent it. After varying periods, some of the abnormally shaped red cells retract their pseudopodia and return to the parent form; others retain their bizarre shapes permanently. Refrigeration (0 C.) prevents the return of the cell from sickle shape to the form of the biconcave disk. Sickle cells kept at body temperature eventually disintegrate by fragmentation.

30. Rous, P., and Robertson, O. H.: Normal Fate of Erythrocytes: I. The Findings in Healthy Animals, J. Exper. Med. **25**:651, 1917; Normal Fate of Erythrocytes: II. Blood Destruction in Plethoric Animals and in Animals with a Simple Anemia, ibid. **25**:665, 1917.

ANAPHYLAXIS IN THE WHITE RAT AS INFLUENCED BY DIET *

BEATRICE CARRIER SEEGAL, M.D.
AND
DEBORAH KHORAZO, M.D.
NEW YORK

It has, in general, been impossible to produce anaphylactic shock in the white rat by the usual procedures employed in such animals as the guinea-pig and the rabbit. Among the early studies on anaphylaxis in this animal, the experiments of Trommsdorf [1] are among the most extensive. He was entirely unable to obtain symptoms of shock in the rat. His results are in agreement with those of Uhlenhuth,[2] Rosenau and Anderson [3] and Galli-Valerio.[4] The latter, however, used *Mus rattus* and *Mus decumamus*. On the other hand, Arthus [5] reported that he was able to produce anaphylaxis in white rats. He promised that details of the procedure would be published in the future, but so far as can be determined they have not appeared. More recently, Novy and De Kruif,[6] as well as Longcope,[7] have reaffirmed the refractoriness of white rats to anaphylactic shock. Opie [8] was unable to reproduce the Arthus phenomenon in these animals.

In contradistinction to this considerable accumulation of negative results, Parker and Parker [9] were able consistently to produce

* Submitted for publication, Jan. 4, 1929.

* From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

1. Trommsdorf, R.: Ueber biologische Eiweisdifferenzierung bei Ratten und Mausen, Arb. a. d. k. Gsndhtsamte. **32**:560, 1909.

2. Uhlenhuth and Weidanz: Ausführung des biologischen Eiweiss Differenzierungsverfahrens, Jena, Gustav Fischer, 1909; Centralbl. f. Bakteriol. (pt. 1, ref., suppl.) **47**:68, 1910.

3. Rosenau, M. J., and Anderson, G. F.: A Study of the Cause of Sudden Death Following the Injection of Horse Serum, Hygienic Laboratory Bulletin, no. 29, U. S. Pub. Health Service, Washington, D. C., 1906.

4. Galli-Valerio, B.: Peut-on utilizer Mus rattus pour le diagnostic des taches de sang par le procide l'anaphylaxie, Ztschr. f. Immunitätsforsch. u. exper. Therap. **5**:659, 1910.

5. Arthus, M. M.: Injections répétés de sérum de cheval chez le lapin, Compt. rend. Soc. de biol. **55**:817, 1903.

6. Novy, F. G., and De Kruif, P. H.: IX. Specific Anaphylactic Shock, J. Infect. Dis. **20**:776, 1917.

7. Longcope, W. T.: Sensitization and Anaphylactic Shock, J. Exper. Med. **36**:627, 1922.

8. Opie, E. L.: Inflammatory Reactions of the Immune Animal to Antigen and Its Relation to Antibodies, J. Immunol. **9**:231, 1924.

9. Parker, J. T., and Parker, F.: Anaphylaxis in the White Rat, J. Med. Research. **44**:263, 1924.

anaphylactic shock in the white rat. Three of twenty-eight animals sensitized to sheep serum died on reinjection of the antigen, nine had severe shock and all but one showed definite symptoms. Ebert,[10] repeating their technic, was unable to obtain their results. Only one of twenty-nine animals showed definite symptoms as described by Parker and Parker.

Two recent workers have succeeded in producing shock in white rats under special conditions. Flashman,[11] while unable to produce shock in normal rats, was successful in those from which the suprarenal glands had been removed. Wedgewood and Grant[12] reported that while animals on a normal diet were immune to anaphylactic shock, rats on a diet deficient in vitamin B could be killed on the second injection of the antigen. The observations of Sartori[13] and of Sereni[14] are related to this problem of the effect of diet on anaphylaxis. These investigators reported that guinea-pigs on a diet deficient in vitamins are more acutely sensitized, and this after a shorter incubation period, than the control animals.

As there was reason to believe from contemporary reports from the animal house that the rats of Parker and Parker were maintained chiefly on a diet of bread and water, which is distinctly deficient in all the vitamins including vitamin B, which Wedgewood and Grant have identified as necessary to the rat's resistance to anaphylactic shock, we planned a series of experiments to test the effect of a dietary of bread and water on anaphylaxis in the rat.

EXPERIMENTS

Anaphylaxis in Animals on Complete Diet.—Using the same technic as reported by Parker and Parker, we endeavored to sensitize the white rat with the following antigens: sheep serum, rabbit serum, rabbit red cells, pigeon red cells and suspensions of killed streptococci. The animals either failed to show any signs of shock on reinjection of the antigen or exhibited only transitory and rather indefinite signs, such as slight ruffling of the hair, a temporary increase in the depth of respiration and occasionally some decrease in muscle tone, as indicated by

10. Ebert, M. K.: Zur Frage des Zustandekommens des anaphylaktischen Shocks bei weizen Ratten, Ztschr. f. Immunitätsforsch. u. exper. Therap. **51**:79, 1927.

11. Flashman, D. H.: The Effect of Suprarenalectomy on Active Anaphylactic Shock in the White Rat, J. Infect. Dis. **38**:461, 1926.

12. Wedgewood, P. E., and Grant, A. H.: The Chemical Basis of Immunity: I. The Influence of Vitamin B upon Anaphylaxis, M. Bull. Univ. of Cincinnati **2**:172, 1924.

13. Sartori: Anafilassi ed avitaminosi, Pathologica **17**:160, 1925.

14. Sereni, E.: Anafilassi ed avitaminosi, Boll. d. Soc. ital. di biol. sper. **2**: 254, 1927.

flabbiness of the animal when handled. Gross and microscopic changes were not found at autopsy.

Fourteen animals were used in this experiment, and the dose of the antigen that caused shock was injected as many as twenty-four times, some of the animals being given injections repeatedly. The diet of these rats consisted of bread and water, oats, carrots, cheese and lettuce.

Anaphylaxis in Animals on a Diet of Bread and Water.—Thirty-eight animals, twenty-four of which had been sired by the same father and raised in the laboratory, were divided into two groups. Sixteen were maintained on a diet of bread and milk, oats and carrots to which cabbage or lettuce was frequently added. The rest were put on a diet of white bread and water, after they had attained a weight of 100 Gm. or more. They were maintained on this diet at least two weeks before the sensitizing dose of antigen was given. On this diet, the animals appeared to be normal, but gained weight only slowly. All the animals were kept in the back of the room, where they were not exposed to any direct sunlight.

All the animals were prepared by a single intraperitoneal injection of sheep serum. The dose was usually 1 cc., although an occasional large animal was given as much as 2 cc. The shocking dose of sheep serum was also 1 cc., given nine or more days later, either intravenously or intracardially. The animals were usually kept for repeated shocking doses at similar time intervals. They were ultimately examined post mortem and the tissues prepared for histologic examination, except for a few animals still under observation.

Many of the animals were bled, from 0.5 to 1 cc. being withdrawn from the heart under ether anesthesia, previous to the administration of the shocking dose of antigen. The serum from this blood was titrated for its precipitin, and in a few cases for its precipitinogen content.

The original toxicity of the antigen was tested by the intravenous injection of 2 cc. or more into animals of both groups. Symptoms were not elicited. Serum from the same sheep was used throughout the experiments. Specificity of the anaphylactic shock and desensitization were also demonstrated.

The results are given in detail in tables 1 and 2. Animals on a diet of white bread and water were subject regularly to symptoms of anaphylactic shock in a manner analogous to that described by Parker and Parker. There is little to add to their description or to that of Flashman, who obtained similar results in rats from which the suprarenal glands had been removed. In about three minutes, the animal's respirations become labored and the abdomen, at the points of insertion of the diaphragm, is retracted markedly with each inspiration. Inspiration often becomes audible and always sounds harsh through the stethoscope. There is frequently a blood-tinged discharge from the nose. The eyes are exophthalmic and the ears, in the severer cases, become pale. The animal flattens itself on its belly and sometimes actually sprawls out on its entire ventral surface with all four legs extended. It may become intensely cyanotic.

TABLE 1.—*The Active Sensitization to Sheep Serum of Animals on a Diet of White Bread and Water*

Rat	Date	Weight, Gm.	Days on Diet	Days Since Last Injection	Amount Injected, Cc.	Place of Injection	Degree of Shock*	Gross Pathologic Changes*	Titer of Precipitin	Minimal Temperature, F.
1	June 16	132	17	..	1	Peritoneal cavity				
	June 25	135	26	9	1	Heart	++	..	....	95
	July 9	140	40	14	1	Tail vein	+	..	....	102.7
	July 20	145	51	11	1	Tail vein	0	..	....	102
	July 28	...	59	8	1	Heart	0	0	....	100.4
2	Sept. 22	218	53	..	2	Peritoneal cavity				
	Nov. 4	...	86	33	2	Foot vein	++	..	....	97.5
5	June 16	152	17	..	1	Peritoneal cavity				
	June 25	154	26	9	1	Heart	+++	+++	....	95.5
6	June 14	199	15	..	1	Peritoneal cavity				
	June 23	188	24	9	1	Heart	++	..	....	96.4
	July 3	182	34	10	1	Vein	++	..	....	96.9
	July 13	180	44	10	1	Vein	++	+	....	97.1
7	June 14	125	15	..	1	Peritoneal cavity				
	June 23	106	24	9	1	Heart	++	..	....	96
10	May 25	119	17	..	1	Peritoneal cavity				
	July 4	...	27	10	1	Vein	+++	±	....	95.1
11	May 25	127	17	..	1	Peritoneal cavity				
	July 5	126	28	11	1	Heart	+	..	....	99.7
	July 14	...	37	9	0.6	Subcutaneous				
	July 24	103	47	10	1	Heart	++	+	....	96.6
12	May 25	134	17	..	1	Peritoneal cavity				
	July 5	133	28	11	1	Heart	++	0	....	96.3
13	July 29	129	33	..	1	Peritoneal cavity				
	Aug. 7	145	42	9	1	Tail vein	++±	..	....	96.4
	Aug. 17	...	52	10	1	Heart	++±	++	1:40	97
14	Aug. 4	110	30	..	1	Peritoneal cavity				
	Aug. 14	113	40	10	1	Vein	++±	..	....	97.2
	Aug. 27	...	53	13	1	Heart	+++	++	1:20	95
15	Aug. 20	137	55	..	1	Peritoneal cavity				
	Aug. 30	...	65	10	1	Heart	++	+	....	96
16	Aug. 14	100	49	..	1	Peritoneal cavity				
	Aug. 23	...	58	9	1	Heart	++	..	1:10	96
	Sept. 7	...	73	15	1	Heart	+++	+++±	1:40	92
17	Oct. 2	...	96	..	2	Peritoneal cavity				
	Nov. 4	...	129	33	1	Foot vein	++	..	....	96.9
18	Aug. 1	103	27	..	1	Peritoneal cavity				
	Aug. 11	109	37	10	1	Heart	+	..	1:10	98.3
	Aug. 22	...	48	11	1	Heart	++	..	1:10	96
	Sept. 5	...	62	14	1	Foot vein	++±	++	1:40	94.7
19	Aug. 4	118	30	..	1	Peritoneal cavity				
	Aug. 14	...	40	10	1	Vein	++	..	1:20	96.5
	Aug. 17	...	43	3	1	Heart	Hemorrhage from heart	..	1:40	
28	Aug. 14	118	40	..	1	Peritoneal cavity				
	Aug. 23	...	49	9	1	Heart	Ditto	+	1:80	96
29	Sept. 18	122	75	..	1	Peritoneal cavity				
	Sept. 27	...	84	9	1	Vein	++	..	0	95.5
	Oct. 17	...	104	20	1	Heart	++	..	....	96.5
	Oct. 26	145	113	9	1	Foot vein	+++	..	....	93.9
	Nov. 16	...	134	21	0.5	Vein				
					0.5	Subcutaneous	+±	..	1:100	98
30	Aug. 21	100	47	..	1	Peritoneal cavity				
	Aug. 30	...	56	9	1	Heart	+	..	1:10	98
	Sept. 10	110	67	11	1	Heart	++	..	1:40	96.6
	Sept. 20	115	77	10	1	Foot vein	+±	..	1:20	97.2
	Oct. 23	135	106	29	1	Foot vein	+	..	....	100.4
	Nov. 14	...	125	19	1	Foot vein	+±	..	1:10	98.6
31	Sept. 19	125	76	..	1	Peritoneal cavity				
	Sept. 29	...	85	9	1	Foot vein	+	..	....	98
	Oct. 26	130	113	28	1	Foot vein	+	..	....	99
	Nov. 9	...	127	14	1	Vein	++	..	1:1280	94.2

* Under the column headed "Degree of Shock" + indicates change in respiration rate and ruffling of hair without prostration or lowering of temperature; ++ indicates the same and, in addition, lowering of the temperature by at least 3 degrees Fahrenheit, and slight prostration; +++ indicates the same as ++ with marked prostration; ++++ indicates the same as +++ followed by death. Similar marks in the column headed "Gross Pathologic Changes" give a rough indication of the extent of the petechial hemorrhages.

TABLE 1.—*The Active Sensitization to Sheep Serum of Animals on a Diet of White Bread and Water.—Continued*

Rat	Date	Weight, Gm.	Days on Diet	Days Since Last Injection	Amount Injected, Cc.	Place of Injection	Degree of Shock	Gross Pathologic Changes	Titer of Precipitin	Minimal Temperature, F.
32	Aug. 2	98	24	..	1	Peritoneal cavity				
	Aug. 11	112	33	9	1.2	Heart	+++	++	....	96
33	Aug. 1	92	21	..	1	Peritoneal cavity				
	Aug. 11	92	31	10	1	Heart	++++	++++	....	96
36	July 28	98	17	..	1	Peritoneal cavity				
	Aug. 6	...	26	9	1	Heart	±	..	....	97.8
	Aug. 22	102	42	16	1	Heart	++	..	1:10	96
	Sept. 5	...	56	14	0.3	Vein				
					0.4	Subcutaneous	++	..	1:40	94.9
	Sept. 18	...	69	13	0.3	Vein				
					0.5	Peritoneal cavity	++	..	1:40	93.1
	Sept. 27	...	78	9	1	Vein	++	..	....	93.4
	Oct. 26	130	107	29	1	Vein	+	..	....	98.1
	Nov. 9	...	120	13	1	Foot vein	++	..	1:20	95

TABLE 2.—*Active Sensitization to Sheep Serum of Animals on Diet of Oats, Bread, Milk and Carrots*

Rat	Date	Weight, Gm.	Days Since Last Injection	Amount Injected, Cc.	Place of Injection	Degree of Shock*	Gross Pathologic Changes*	Titer of Precipitin	Minimal Temperature, F.
Pn. 4	June 25	...	..	1.9	Tail vein				
	July 9	130	14	1	Tail vein	+	0	....	101.2
4	June 18	162	..	1	Peritoneal cavity				
	June 27	153	9	1	Tail vein	+	..	....	100.8
8	June 12	120	..	1	Peritoneal cavity				
	June 21	...	9	1.2	Heart	0	0		
9	June 12	129	..	1	Peritoneal cavity				
	June 21	...	9	0.9	Tail vein	+	0		
21	July 29	120	..	1	Peritoneal cavity				
	Aug. 17	...	19	1	Peritoneal cavity	0	..	1:5	
	Aug. 27	148	10	1	Heart	++	+	1:10	95.5
23	Aug. 14	...	..	1	Peritoneal cavity				
	Aug. 23	158	9	1.2	Vein	0	..	0	100.6
25	Aug. 20	148	..	1	Peritoneal cavity				
	Aug. 30	...	10	1	Foot vein	+	..	0	99.8
	Sept. 10	165	10	1	Tail vein	0	..	1:40	99.3
	Sept. 20	165	10	1	Foot vein	0	..	1:15	98.8
	Oct. 29	...	39	1	Peritoneal cavity				
	Nov. 14	...	16	0.7	Foot vein				
				0.3	Subcutaneous	±?	..	1:40	98.7
25	Aug. 21	160	..	1	Peritoneal cavity				
	Aug. 30	...	9	1	Heart	±	..	0	99
	Sept. 11	200	11	1	Peritoneal cavity	0	..	1:10	
	Sept. 20	215	9	0.7	Tail vein	..	..	1:40	98.2
				0.3	Subcutaneous	0			
	Oct. 29	...	39	1	Foot vein	0			
	Nov. 14	...	16	0.7	Tail vein	±?	..	1:20	100
				0.3	Subcutaneous				
27	Sept. 18	225	..	1.7	Peritoneal cavity				
	Sept. 27	...	9	1	Foot vein	0	..	1:5	
	Oct. 29	...	32	1	Foot vein	0			
	Nov. 14	...	16	0.5	Tail vein				
				1	Subcutaneous	0	..	1:40	100
34	Sept. 19	140	..	1	Peritoneal cavity				
	Sept. 29	...	10	1	Foot vein	++	..	1:40	95.1
	Oct. 26	130	27	1	Foot vein	+	..	....	99.4
	Nov. 9	...	14	1	Vein	+	..	1:80	100
Im. I.	Sept. 10	...	..	2	Vein				
	Sept. 20	310	10	2	Tail vein	0	..	....	99.6

* For meaning of signs, see table 1.

In the severe cases, general flaccidity develops. The animal is limp, and all reflexes disappear. The respirations become shallow and rapid. Tonic and clonic convulsions and opisthotonos occur intermittently. One animal excreted a bloody urine, and several animals had a bloody discharge from the rectum.

As will be seen from table 1, among the animals on bread and water, one animal died, eight showed marked signs of shock at one time or another, and all but one exhibited distinct signs of shock. The drop in temperature was a pronounced and constant feature of the shock, amounting to from 3 to 10 F.

In table 2, detailed information is given concerning the eleven animals on a complete diet. These animals constitute a control group for the series described in table 1. Two animals showed distinct signs of shock with a drop in temperature at one time, five showed slight, questionable reactions to the antigen, without a drop in temperaure, and four did not show any reaction. Five other animals were lost as the result of hemorrhage from the heart.

PATHOLOGY

With two exceptions, those animals on a diet of bread and water that were examined post mortem following anaphylactic shock showed a uniform pathologic picture, varying only in extent and intensity. Petechial hemorrhages in the viscera were the outstanding feature, as already reported by Parker and Parker.[9] The mesenteric lymph nodes were the first organs to exhibit hemorrhage, while the liver and the gastro-intestinal tract were the next most common sites. Peyer's patches were only occasionally involved. There was often free blood in the stomach and small intestine. In the severer cases, minute hemorrhages in the pancreas, kidneys and lungs could be demonstrated. These varied from pin point, almost submacroscopic size, to a size about 2 mm. in diameter. The lungs, at the stage at which these animals were examined post mortem, were not emphysematous.

One of the animals on a full diet which were examined post mortem showed two small hemorrhages in two of the mesenteric lymph nodes; the others were, in the gross, normal.

In view of the fact that Flashman produced anaphylaxis in rats from which the suprarenal glands had been removed, it is interesting to note the difference in the size of these glands in the two series of animals. The suprarenal glands taken after autopsy were measured in the three dimensions. By this rather crude method, the suprarenal glands of the animals on bread and water were ascertained to be approximately one half the size of the suprarenal glands of the animals on a complete diet, while the average body weights of the animals in these two series differed by only 17 Gm., the one series averaging 141 Gm., the other 124 Gm. This difference in size could not be

explained on the basis of sex. Female rats were about equally distributed in both groups.

Rat 1, which failed to show an anaphylactic response two times out of four, was the only animal of the series on bread and water that had suprarenal glands comparable in size with those of the control group, while the smallest suprarenal glands were found in rat 33, female, which died of shock.

These observations suggest that the two sets of animals, those on complete diet and those on bread and water, may not differ in their fundamental response to the foreign protein, sheep serum, but may differ in some protective mechanism supplied by the secretions of the suprarenal glands.

A more complete gross study and a histologic study of these animals will be reported at a later time.

TABLE 3.—*Precipitinogen Content of the Serum of White Rats Sensitized to Sheep Serum*

Rat	Total Amount of Sheep Serum Injected, Cc.	Amount of Last Injection, Cc.	Days Since Last Injection	Highest Dilution Giving Ring
27	1.8	1.8	0	1:160
34	1	1	10	1:80
	3	1	14	1:40*
35	1	1	10	1:160
	3	1	14	1:12*
Im. I.	12	2	6	1:640
29†	1	1	9	1:320
31†	1	1	10	1:160
	3	1	14	0
36†	7	1	14	1:20*

* Rings faint and rather inconclusive.
† The last three animals belong to the series on a diet of bread and water.

PRECIPITIN AND PRECIPITINOGEN CONTENT OF THE SERUMS

The precipitin and precipitinogen content of the rats' serums was determined by means of the ring test in small tubes made from glass tubing with an inside diameter of 3 mm. The readings were taken after one and a half hours' incubation at 37 C. Appropriate control tubes with sodium chloride solution and with serum from normal rats, both on a complete and on a bread and water diet, were also prepared. It was found advisable in determining the titer of the precipitin to dilute the rats' serums 1:2 because of the frequency of a ring when physiologic solution of sodium chloride was layered over undiluted rat serum. The numbers given in the table under the heading "Titer of Precipitin" represent the highest dilutions of sheep serum antigen to give a ring. The titer is actually somewhat higher, as the rat serum had been diluted as mentioned. With the exception of rat 31 from the series on bread and water, the serum of which showed a titer of precipitin far in excess of the titers of precipitin of the serums of the other animals, the two

series did not show any striking difference in the titers of the precipitin of their serums. Formation of precipitin in the rat is therefore not marked, as has already been reported by Longcope [7] and by Spain and Grove.[15]

The precipitinogen content of the serum was determined in only a few rats by means of a strong, undiluted rabbit antisheep serum, which, when tested with sheep serum, gave a titer of 1:25,000 by the ring method described. Table 3 details the results. The sheep serum had almost disappeared from the rats' circulation at the end of fourteen days, which agrees with Longcope's observations. Evidence of a difference between the two series of animals in the precipitinogen content of their serums was not presented in these few experiments.

SUMMARY

Pronounced symptoms of anaphylactic shock cannot be produced in white rats on a diet of oats, bread, milk and greens.

Symptoms of anaphylactic shock can be constantly produced in white rats on a diet of white bread and water.

The gross lesions of anaphylaxis in the white rat are small petechial hemorrhages into various viscera, notably the mesenteric lymph nodes, the liver, the gastro-intestinal tract, the kidney, the pancreas and the lung.

The suprarenal glands of rats on a diet of bread and water are considerably smaller than those of animals on a complete diet.

So far as investigated, the titer of the precipitin and the titer of the precipitinogen content of the serum of sensitized rats on a diet of bread and water do not differ significantly from the corresponding titers of the serum of sensitized animals on a diet of oats, greens, bread and milk.

15. Spain, W. C., and Grove, E. F.: Studies in Specific Hypersensitiveness: XII. A Study in Rat Precipitin, J. Immunol. **10**:433, 1925.

Laboratory Methods and Technical Notes

A SIMPLE GLYCEROL WATER CRYSTAL VIOLET POTATO CYLINDER MEDIUM FOR DIAGNOSTIC CULTURES OF TUBERCLE BACILLI*

H. J. Corper, M.D., and Nao Uyei, Ph.D., Denver

In previous communications,[1] results were reported which led to a new method for the isolation and cultivation of tubercle bacilli from contaminated tuberculous materials. In a later report,[2] it was demonstrated that the new method is superior to Petroff's method of isolation and equals in efficiency the method that employs inoculation of guinea-pigs for the detection of the presence of tubercle bacilli in urines, sputums, tissues and other contaminated materials, and in many respects surpasses the latter method for practical diagnostic purposes.[3]

Further observations on the urine in doubtful cases and experiments with pure tubercle bacilli in salt solution have confirmed the previous observations demonstrating the efficiency of the new culture method as compared with the method that employs inoculation of guinea-pigs.

In one of the earlier reports,[2] it was pointed out that crystal violet, when incorporated in a medium of mashed potato and agar, exerted a deleterious effect on the growth of the tubercle bacilli. This was further confirmed on more extensive study. Therefore mediums made of mashed potato and agar cannot be recommended for use in the isolation of tubercle bacilli. When, however, it is desired to take advantage of the growth-promoting properties of potato incorporated in a solid medium similar to an agar medium for growing pure transplants of tubercle bacilli, the following simple medium is advised, with the omission of the crystal violet and broth.

Mashed autoclaved potato	25	per cent by weight
Glycerol	2.5	per cent by weight
Agar-agar	1.5	per cent by weight
Distilled water	71	per cent by weight

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* From the Research Department, National Jewish Hospital.

1. Corper, H. J., and Uyei, Nao: The Isolation of Tubercle Bacilli from Contaminated Tuberculosis Materials, Am. Rev. Tuberc. **16**:299, 1927; The Cultivation of Tubercle Bacilli: An Improved Method for Isolation from Tuberculous Materials, J. Lab. & Clin. Med. **13**:469, 1928.

2. Corper, H. J., and Uyei, Nao: Further Observations with a New Method for Cultivating Tubercle Bacilli: A Comparison with Guinea-Pig Inoculation and Petroff's Method, J. Lab. & Clin. Med. **14**:393, 1929.

3. Corper, H. J.: The Certified Diagnosis of Tuberculosis, J. A. M. A. **91**: 371 (Aug. 11) 1928.

This nutrient medium cannot, however, replace the crystal violet potato cylinder medium for the isolation of tubercle bacilli from contaminated tuberculous materials.

Note was made in our earlier reports that broth added to the potato cylinder medium used in the new culture method, played a more or less indifferent rôle in promoting the growth of the tubercle bacilli on this medium. The possibility of obviating the use of broth in the potato medium was therefore suggested. In order to test this point more carefully before the omission of broth from the medium was recommended, potato cylinder crystal violet mediums were prepared containing varying amounts of pure glycerol in aqueous solution, and their efficiency for use in the growing of tubercle bacilli was compared with the medium previously recommended, which contained crystal violet

TABLE 1.—*Crystal Violet Potato Cylinders in Glycerol Water Compared with Crystal Violet Potato Cylinders in Glycerol Broth as Mediums for the Growth of Tubercle Bacilli*

Amount of Glycerol Solution in Which Crystal Violet Potato Cylinder* Was Contained	Amount of Virulent Human Tubercle Bacilli in Milligrams per Cubic Centimeter in Suspension Used for Seeding Culture Tubes, with Growth Resulting		
	1 Mg. per Cc.	0.0001 Mg. per Cc.	0.000,001 Mg. per Cc.
5% glycerol broth	Luxuriant	Marked	Marked
5% glycerol water	Luxuriant	Marked	Marked
4% glycerol water	Marked	Marked	Marked
3% glycerol water	Marked	Appreciable	Appreciable
2% glycerol water	Marked	Appreciable	Not appreciable
1% glycerol water	Appreciable	Appreciable	Not appreciable

* The potato cylinders were prepared by immersing the cleancut cylinders in a freshly mixed solution of 0.0015 per cent crystal violet in 1 per cent sodium carbonate.

potato cylinders in 1.5 cc. of 5 per cent glycerol broth. The results of this test are recorded in table 1.

In order further to test the value of the glycerol water in replacing the glycerol broth in the preparation of the crystal violet potato cylinder medium used for detecting tubercle bacilli in tuberculous materials, comparative studies were made in which from 1 to 7 per cent glycerol water was tested, positive sputums being used as test material and 5 per cent glycerol broth crystal violet potato cylinder medium as control. The sputums were prepared for culture by treatment with an equal volume of 6 per cent sulphuric acid, as recommended in the previous reports. The results of this study in the isolation of tubercle bacilli from sputums are recorded in table 2.

It is to be noted from an examination of the data recorded in tables 1 and 2 that glycerol water, containing glycerol in strengths of from 4 to 7 per cent, added to the crystal violet potato cyclinders yielded growths of tubercle bacilli equally well and with as few contaminations as did the 5 per cent glycerol broth crystal violet potato cylinder

medium. Similar results were obtained when other tuberculous materials were used for the test and when bovine tubercle bacilli were used.

Since the use of glycerol water in place of glycerol broth markedly simplifies the preparation of the medium, the sulphuric acid potato method for the isolation and detection of tubercle bacilli consists essentially in the following procedures:

One cubic centimeter of suspected material is beaten to a homogeneous pulp and introduced into a sterile centrifuge tube of 15 cc. capacity with 1 cc. of 6 per cent sulphuric acid (containing 17 cc. of 96 per cent [specific gravity, 1.84] sulphuric acid in 500 cc. of distilled water) and mixed. After incubation at 37 C. for thirty minutes, the contents of the tube are mixed with about 10 cc. of sterile

TABLE 2.—*Comparison of Glycerol Water Crystal Violet Potato Cylinder Medium with Glycerol Broth Crystal Violet Potato Cylinder Medium for Cultivating Tubercle Bacilli from Sputum*

Amount of Glycerol Solution in Which Crystal Violet Potato Cylinder† Was Contained	Results Obtained, in Percentage of Tubes Planted*	
	Contaminations, per Cent	Isolations, per Cent
5% glycerol broth (control)	4	93
7% glycerol water	1	99
6% glycerol water	0	99
5% glycerol water	3	96
4% glycerol water	3	97
3% glycerol water	5	88
2% glycerol water	3	89
1% glycerol water	3	84

* In this table are recorded the results of the isolation of tubercle bacilli from fifteen specimens of sputums that were positive in stained smears examined under the microscope, five tubes of medium being used for the testing of each specimen of sputum, making a total of seventy-five tubes from which the percentage of the contaminations and the isolations in the respective columns were figured.

† The percentage of glycerol recorded is the amount contained in the 1.5 cc. of the solution placed in the culture tube in which the potato cylinder rested.

0.9 per cent sodium chloride solution and centrifugated. The residue, after the supernatant fluid has been decanted, is seeded on the surface of the glycerol water crystal violet potato cylinder medium, the culture tube being capped with tin foil after the cotton plug has been lightly impregnated with hot paraffin to prevent drying out of the medium. The medium is prepared by placing 1.5 cc. of 6 per cent aqueous solution of glycerol (made with pure tap water or distilled water) in a sterile culture tube, 6 inches by ¾ inch (15.24 by 1.9 cm.) in size, in which has been inserted the crystal violet potato cylinder, about 3 inches (7.6 cm.) long and ⅝ inch (1.59 cm.) in diameter. The latter is made by soaking a clean potato cylinder halved longitudinally, in a freshly mixed 0.0015 per cent standard crystal violet in 1 per cent sodium carbonate solution (prepared from the pure anhydrous salt). The entire medium is sterilized in an autoclave at 15 pounds (6.8 Kg.) pressure for thirty minutes. Excessive or prolonged heating of the medium during sterilization is to be avoided. The culture tubes should be incubated in the dark with due precaution being taken to avoid drying of the medium or contamination. A luxuriant growth should occur on this medium within from two to six weeks; but if the culture is negative, the tubes should not be discarded for diagnostic purposes until after three months' observation at incubator temperature.

SUMMARY

In the sulphuric acid potato culture method for the diagnosis of tuberculosis, 6 per cent glycerol water can be used to replace the 5 per cent glycerol broth previously recommended for the preparation of the crystal violet potato cylinder medium. This simplifies the preparation of the culture medium used in this new diagnostic method.

Human and bovine tubercle bacilli grow with equal facility when present in small numbers in tuberculous materials on the crystal violet potato cylinder medium with glycerol water in the absence of broth.

General Review

HUMAN PALEOPATHOLOGY

WITH SOME ORIGINAL OBSERVATIONS ON SYMMETRICAL OSTEOPOROSIS OF THE SKULL *

HERBERT U. WILLIAMS, M.D.
BUFFALO

CONTENTS

* Submitted for publication, Jan. 14, 1929.
* From the University of Buffalo, School of Medicine.

The word paleopathology was coined by Ruffer [1] as a name for the science of disease in persons of ancient times. The pursuit of any branch of knowledge for its own sake does not need justification; however, it is manifest that paleopathology may be of use in helping to explain diseases of the present time. It may, for instance, throw light on the origin of some of the infections. Again, the contrast between the diet and habits of primitive men and those of civilized men opens a field for study of diseases of the teeth that may yield results of practical value to men of the present day.

The monographs, papers and abstracts from which this review has been compiled have been found, for the most part, in journals of archeology, ethnology and anthropology, and in the reports of surveys, of museums and of learned societies in various parts of the world. As such sources of information are not easily accessible to pathologists, it is hoped that the review may be useful to them. Obviously, some valuable material may have been overlooked; furthermore, new material is coming to light almost daily.

SOME CASES OF IDENTIFICATION

Precisely where, in point of time, the paleopathology of man begins and ends is not easy to define, nor is it a matter of great moment. The case of Admiral John Paul Jones, distinguished for his services to the American colonies during the war of the Revolution, may not belong to ancient history, but it is sufficiently outside the routine experience of pathologists to be worth recalling.

Jones was buried in Paris in 1792, the body being enclosed in a coffin of lead containing alcohol. In 1905, when his remains were removed for transportation to the United States, even the features of the face were found corresponding exactly with a bust made during his life. The identification by anthropologists was supplemented by an autopsy that seems to have given satisfactory results.[2]

The lungs, heart, liver, stomach, spleen and kidneys were in the main well preserved. A scar in the left lung was attributed to an attack of pneumonia that Jones was known to have had three years prior to his death and which was said by physicians in Paris a year later to have affected his left lung permanently. The striations of the cardiac muscle were visible. The spleen was large and firm. The liver was yellowish brown and somewhat contracted; the liver cells were not well preserved. Some of the vessels of the kidney were sclerotic and the

1. Ruffer, M. A.: Path. & Bact. **18:** 149, 1913; Paleopathology of Egypt, Chicago, University of Chicago Press, 1921, p. 139.

2. Porter, Horace: The Recovery of the Body of John Paul Jones, Century Magazine **70:** 948, 1905.

glomeruli were in part fibrous. Bacteria were demonstrated in the liver and the kidneys. A diagnosis of "interstitial nephritis" was made, and it was stated that the results of the autopsy were consistent with the history, the patient having had severe dropsy for a week or more before his death. The report on the histologic changes and its conclusions were confirmed by Cornil. The excellent preservation of this body for 113 years was doubtless due to the alcohol. The odor of alcohol was still evident in spite of a crack in the coffin.[3]

Another case in which the condition of the body assisted in its identification was that of Don Francisco Pizarro, whose remains were exhumed at Lima, Peru, in 1891, on the 350th anniversary of his death. Pizarro was known to have been assassinated, and the wounds found on the body, in particular one on the right side of the neck, corresponded with those described in the accounts of his tragic end. Apparently, embalming had not been done, but the muscles, tendons and vessels could be traced, and the right eye was recognizable. The face in general and the viscera were not well preserved.[4]

An interesting, if not entirely convincing, story is related by Fürst [5] of an Icelandic tradition to the effect that King Olav Geirstadaalv, of the ninth century, was a tall man who died having a disease of the foot. A great mound said to have been built by this king yielded the skeleton of a tall man whose bones were deformed by "rheumatism," especially one foot and the left knee.

Among the many dramatic episodes brought to light in the study of Egyptian mummies is one of exceptional interest related by Elliott Smith.[6] An explorer of Egyptian antiquities had searched for the mummy of Queen Tiy, wife of the Pharaoh Amenophis III, of the eighteenth dynasty, and he thought he had found it. Other Egyptologists maintained that the archeologic evidence proved that the mummy was that of the heretic King Akhenaton (Amenophis IV). The bones were submitted to Smith as those of the queen. To his surprise, he was forced to the conclusion that the skeleton was that of a young man and not that of an old lady. However, the condition of the epiphyses pointed to an age of about 23 years, certainly not more than 30 years, if

3. It would be interesting to know whether or not this incident inspired an amusing, if somewhat irreverent, song, popular among college students a few years ago.

4. McGee, W. J.: Remains of Don Francisco Pizarro, Am. Anthrop. **7**: 1, 1894.

5. Fürst, C. M.: När de döda vittna, Stockholm, Svenska Teknologföreningens (Tissell's) förlag, 1920; abstr., Anthropologie **33**:605, 1923.

6. Smith, G. E.: Cambridge Univ. Med. Soc. Mag., 1926, p. 32. Smith, G. E., and Dawson, W. R.: Egyptian Mummies, London, George Allen & Unwin, 1924; The Broadway, Westminster Hosp. Gaz. **4**:25, 1928.

the owner of the skeleton was normal. The historical evidence indicated that the king was at least 30 years old and probably 36 when he died. It happened that a number of contemporary portraits of Akhenaton had come down to these times. His feminine appearance in these portraits had long been remarked. As his skull indicated a slight degree of hydrocephalus, and his lower jaw was somewhat overgrown, an additional sign of disease of the hypophysis, Smith [6] suggested that Akhenaton may have been a victim of dystrophia adiposogenitalis, or Froelich's syndrome, which leads to a delayed union of the epiphyses.

From such examples, occurring within historic times, which could be multiplied, human paleopathology may be extended to a time 50,000 or more years earlier when evidences of disease in the bones and teeth of the Neandertal man of the latter part of the glacial period were encountered. The bodies of Eskimos, encased in ice and several centuries old, have been discovered in Alaska, and long-haired mammoths have been found in the frozen mud of northern Siberia, the carcasses of which had been well preserved by the cold for many thousands of years. Though it may seem fantastic, it is not impossible that some pathologist may yet have the thrilling experience of making an autopsy on the frozen or desiccated body of a Neandertal man.

CHRONOLOGY

For the chronology, one must rely on archeologists. Their methods are essentially those of geologists. When strata lie one above another, objects found in the lowest levels should be the oldest. However, there are possibilities of error that have led to many controversies. Burial may introduce a skeleton into a level lower than that to which it belongs (intrusive burial). A stratum formed under water may contain objects that have washed in from an older stratum. An Indian may have picked up the bone of a mastodon, as any one might do; if such a bone were found buried with the Indian, it might lead to the erroneous conclusion that this Indian and this mastodon were contemporaries. Strata formed by recent rains and floods may falsely seem continuous with older strata. I myself extracted an intact beer bottle from gravel that I had supposed dated from the middle of the glacial period. Evidently, the amateur archeologist may easily make mistakes, while professional archeologists may sometimes hold diverse opinions.

Archeologists have projected chronology backward by many thousands of years. The end of the glacial period is the earliest date that can be stated approximately in years, and it may be placed at from 10,000 to 30,000 years ago. Some of the discrepancy in these figures may be explained when one recollects that the glacial period must have come to an end in such a region as the neighborhood of New York

much earlier than it did in such localities as Montreal, about 300 miles farther north. The glacial period has not yet come to an end in Greenland.[7] The early Egyptians may have had a neolithic culture when much of the Scandinavian peninsula was buried in ice.

It appears that in the glacial (or pleistocene) period there were several long intervals during which the ice receded and warm climates prevailed. Penck, of Munich, has recognized in Europe four glacial periods, named Günz, Mindel, Riss and Würm, the first being the oldest. They were separated by three interglacial periods, as the Riss-Würm and so on. Estimates of the lapse of time required for the four glacial periods and the three interglacial periods vary from more than 100,000 to 1,000,000 years.

In North America, there are evidences that the ice sheet advanced and receded as in Europe, but it is not at present possible to coordinate the glacial periods and the interglacial periods of the two continents. It seems expedient to introduce these elementary facts and those that are given in the immediately ensuing paragraphs, since it will be necessary to refer repeatedly to the chronology adopted.[8]

As is well known, archeologists have designated stages in the development of human culture according to the use in them of iron or bronze or polished stone or rough stone. These stages may be arranged in a chronological table, omitting all but general headings, as follows:

Iron age, beginning about 1500 B. C. to 500 B. C.
Bronze age, beginning about 4000 B. C. to 1000 B. C.
Neolithic age, beginning about 10000 B. C.
Paleolithic age, including:

The reindeer period, or period of cave artists, men of modern skeletal type;	subdivided into Magdalenian, Solutréan and Aurignacian	Latter part of the Würm glaciation, from 15,000 to 40,000 years ago.
Neandertal or Mousterian race	Earlier part of the Würm glaciation and the Riss-Würm interglacial period.	

Iron and bronze were employed in southwestern Asia and in Egypt long before they were introduced into western Europe. The oldest objects of iron that I can learn of are some iron beads discovered by Flinders Petrie in a predynastic tomb in Egypt (i.e., of 3400 B.C. or earlier). The tomb of King Tut-ankh-amen (1400 B.C.) contained

7. For a concise and readable account of the glacial period, I refer the reader to Coleman, A. P.: Ice Ages, Recent and Ancient, New York, The Macmillan Company, 1926.

8. There are many excellent works largely devoted to these subjects: MacCurdy, G. G.: Human Origins, New York, D. Appleton & Company, 1924; Osborn: Men of the Old Stone Age, New York, Charles Scribner's Sons, 1918. Boule: Les hommes fossiles, Paris, Masson & Cie, 1923. Obermaier: Fossil Man in Spain, New York, Hispanic Society, 1924.

a fine dagger and other well made articles of iron.[9] Bronze was the metal in use at the time of the Trojan war.

During the bronze age, cremation was practiced in some parts of Europe so that human remains are largely lacking from those regions. It is customary to refer objects found in Egypt to one of the numerous dynasties, the first of which, according to Breasted, had its beginning about 3400 B.C.

The most important event in the chronological table is the appearance of men of the modern skeletal type (including the so-called Cro-Magnon race) in the latter part of the Würm glaciation.

Still, going backward, one finds the transition from modern man to Neandertal man abrupt. The number of skulls and even of fairly complete skeletons of Neandertal man is large enough to make his reconstruction possible. He was short, stocky, powerful, bending at the knees, with his head protruding forward and showing a strong lower jaw and teeth, with chin receding or wanting, a large face, eye-sockets large, eyebrow ridges prominent, forehead receding and a foramen magnum rather far back. His brain was of good size, and the convolutions were, in general, the same as those in modern man, but somewhat simpler. The frontal bones were relatively less developed. He was right-handed. He used fire. He buried his dead, which accounts for the considerable number of finds of skeletal remains. He is the original caveman of fiction, the low-brow of modern slang. A majority of anthropologists appears to believe that Neandertal man was a different species from modern man and that he became wholly extinct. Hrdlička, of the United States National Museum, Washington, has ably defended the position that modern man is descended from Neandertal man, and that this development occurred in central Europe.

The possibility that atavistic and now criminal tendencies inherited from Neandertal man may appear in individuals of the present offers an interesting field for speculation. But it may be that a great injustice has been done Neandertal man; for all one knows, he may have had a mild and gentle disposition, while having a forbidding exterior.

Although numerous stone implements have been found that belong to the middle or early parts of the glacial period, at present only two fragments of human remains are known to have come from those remote times: the Heidelberg or Mauer jaw, which does not show any pathologic condition, and the incomplete Piltdown skull. The latter is so thick as to have suggested that it came from a man with acromegaly[10] or from one with Paget's disease.

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9. Carter, Howard: The Tomb of Tut-ankh-amen, New York, Doubleday, Doran & Company, 1927, vol. 2, p. 248. The examination of the body of King Tut-ankh-amen by Derry did not give results of importance to pathologists.

10. Adami, J. G.: Medical Contributions to the Study of Evolution, New York, The Macmillan Company, 1918.

Shattock[11] made a careful study of this famous skull from the point of view of the pathologist. While he excluded osteitis deformans, acromegaly and several other conditions as causes of the thickening, he seemed to think that it might have been due to rickets, and concluded that, at least, it may have been pathologic. I have had the opportunity to give the Piltdown skull a brief inspection, and was impressed with the fact that it was extremely thick, and that this thickness might be pathologic, but the nature of the material did not permit an exact diagnosis.

In America, it is exceedingly difficult to determine the age of native human remains. The Indians continued to use the implements of their own culture long after the white race came. In fact, in Mexico and in our own Southwest, one may any day see a woman grinding corn in a stone metate within a few yards of automobiles and railroads. The presence in a grave of glass beads or iron axes may prove that the burial was recent, but the absence of such articles does not prove that it was ancient. Superimposed strata containing evidences of different cultures, such as are common in Europe, are rather rare in America. Much progress in defining the sequence of cultures has been made in Peru, in Central America, in the Southwest of the United States and in Ohio, to mention some of the best known fields. Realizing the immense activity of American archeologists, one may look forward to a solution of many of the difficulties before long.

Whether or not there is reliable evidence for the existence of man in America during the glacial period is still, as it has been for many years, a matter of lively controversy. Anthropologists seem fairly agreed that the Indians constitute a well defined race, that their progenitors came from Asia by successive migrations and that their sojourn in America has been a long one. In a previous paper,[12] I suggested that the probable absence from America of most of the infections of worldwide distribution, prior to the time of the discovery, and the great susceptibilty of the Indians to infections introduced from Europe are indications that the separation of the American race from the races of the Eurasian continent has been a long one.

MATERIALS

History contributes to paleopathology facts of the utmost importance, but in the present review only the material sources will be considered. The largest proportion of the facts of human paleopathology has come from the study of ancient bones and teeth. These are the only sources

11. Shattock, S. G.: Tr. XVIIIth Internat. Cong. Med., London, 1913, Sec. on Path., p. 3.

12. Williams, H. U.: Bull. Johns Hopkins Hosp. **20**: 339, 1909.

of evidence for the conditions of disease of the earlier periods of man's history.

The bones of neolithic, late-paleolithic and Neandertal man which show signs of disease are preserved in museums scattered over western and central Europe. The publications in which they are described are scattered in like fashion. Egypt has furnished an enormous amount of material, which dates from the late prehistoric era down to early Christian times. Smith [13] has examined about 30,000 ancient Egyptian and Nubian bodies, while Wood-Jones [13] based his report on the examination of 6,000 bodies: more than all the rest of the world has given to the science of disease in ancient man.

At the time of the enlargement of the great Assuan dam, near the border where ancient Egypt and Nubia met, it was recognized that the region to be flooded contained a vast quantity of priceless archeologic material. Fortunately for science, the study of the human remains was entrusted to Smith, who was assisted by Wood-Jones and Derry. Their labors were begun in 1907, and were completed in 1910, and their final report was given out in 1910. To appreciate the value of their labors, one must realize how time-consuming are the measurements of the skull and skeleton made by physical anthropologists, the personal discomforts attending field work in Nubia and the fact that some of the gentlemen named carried on, at the same time, their regular duties at the medical school in Cairo. The promptness with which their results were given to the world under these conditions and their thorough but concise analysis of that great mass of human remains challenge admiration. It is to be regretted that their reports [13] are not more easily accessible to the general student.

For facts concerning the paleopathology of Egypt, one is also indebted to the studies of Ruffer.[1] Besides these there are numerous less extensive articles on material from Egypt.

In America, one is fortunate in having brief notes on the bones of the North American Indians made early by a competent pathologist, Whitney,[14] as well as the excellent paper of Landon.[15] The largest single source of South American Indian remains has been Peru, and

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13. Wood-Jones, F.: General Pathology, Fractures and Dislocations, in Human Remains, Archeological Survey of Nubia, Report for 1907-1908, Cairo, 1910, vol. 2. Smith, G. E.; Wood-Jones, F.; Derry, D. E.: Articles in Bull. Archeological Survey of Nubia, 1908-1910, nos. 1-6. Smith, G. E., and Dawson, W. R.: Egyptian Mummies, London, George Allen & Unwin, 1924. The latter contains a good brief summary of the pathologic conditions encountered in Egyptian remains.

14. Whitney, W. F.: Diseases of the Bones of North American Indians, Rep. Peabody Museum **3**:433, 1884.

15. Landon, F. W.: Madisonville (Ohio) Pre-Historic Cemetery, J. Cincinnati Soc. Nat. Hist. **4**: 247, 1881.

the most complete reports on the pathologic conditions shown in the bones are those of Hrdlička [16] and MacCurdy,[17] both reports furnished with beautiful illustrations. I am greatly indebted, also, to Moodie [18] for the facts that he has assembled, bearing on both continents. Within a few years, a large quantity of skeletal material of the Pueblo Indians has been excavated at Pecos, N. M., in the United States, by Kidder. The human remains will be the subject of a report by Hooton.[19] Unfortunately, it is not available for the purposes of this review. I had the opportunity of giving some of these bones a brief inspection and found that there are among them cases of symmetrical osteoporosis of the cranium, of fractures, of osteomyelitis, possibly some of tuberculosis, of probable osteomalacia and of thickening of various long bones that might have been produced by syphilis or by periostitis or by osteitis deformans. There are also among them cases of arthritis deformans. Much remains to be done in studying the pathologic conditions of the bones of Indians deposited in the great museums.

TECHNIC

Bones, when first unearthed, are sometimes so soft and fragile that it is desirable to endeavor to preserve them by infiltration with such materials as paraffin or shellac. Sections of bone and teeth may be made by grinding, the technic for which is described in textbooks of histology, so that it need not be repeated here. Sections may be made after bone has been decalcified by the usual methods. Even when a bone is hundreds of years old, it is best to fix it in formaldehyde before decalcification. Some old bones are infiltrated with so much calcium carbonate that decalcification must be done carefully on account of the evolution of carbon dioxide, which tears the tissue. Embedding in celloidin is usually recommended. If the amount of organic framework left is slight, decalcification may be performed after embedding the tissue in celloidin. In one case, I obtained usable sections by this method when decalcification before embedding had reduced the tissue to a formless jelly. A recent monograph from Aschoff's laboratory considers the preparation of ground sections of bone and their examination by transmitted, reflected and polarized light, with especial reference to the study of ancient specimens.[20]

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16. Hrdlička, A.: Pathology of the Ancient Peruvians, Smithsonian Coll. **61:** 57, 1914.

17. MacCurdy, G. G.: Human Skeletal Remains from the Highlands of Peru, Am. J. Phys. Anthrop. **6:**217, 1923.

18. Moodie, R. L.: Paleopathology, Urbana, Ill., University Illinois Press, 1923.

19. Hooton, E. A., to be published by the Phillips Academy, Andover, Mass.

20. Weber, Moritz: Schliffe von Röhrknochen und ihre Bedeutung für die Unterscheidung der Syphilis und Osteomyelitis von der Osteodystrophia fibrosa, sowie für die Untersuchung fraglich syphilitischer prähistorischer Knochen, Beitr. z. path. Anat. u. z. allg. Path. **78:**442, 1927.

OBSERVATIONS ON ANCIENT BONES

Erosion of bones by natural forces after death, including roots of plants and rodent animals, may produce results that simulate the effects of diseases destructive of bone. Elliott Smith [21] discussed errors of diagnosis in the case of certain ancient Egyptian bones that had been gnawed by a small beetle.

Deformations.—Examples of arrested or defective development are not rarely observed in ancient bony remains, such as cleft palate, metopic suture and the presence of an interparietal bone. The latter is said to be frequent in native Peruvian skulls, and is often called the Inca bone. Deformity of the skull may be natural or artificial. Both kinds are widely distributed. The possible relation of asymmetry of the cranium to disease of the nervous system is discussed briefly by Raymond.[22]

Artificial deformation results from pressure on some portion of the head exerted during infancy. Such deformity may have been unintentional, and was common among many American Indians when the occiput of the baby constantly pressed on a resistant support. Intentional deformation of the skull has been practiced in many parts of the world and from early periods. A good brief account of it, with references to the literature, was given by Hrdlička,[23] who said the effects on the health of the subject were insignificant.

Large symmetrical depressions on the parietal bone, which have been attributed to senile atrophy or to congenital defect, and which are usually rare, were found by Smith [24] with great frequency in bodies from ancient Egypt, but only in remains from the upper classes of certain periods and of certain localities. One cemetery at the Pyramids yielded seventy examples. It was suggested that the depressions were produced by the wearing of heavy wigs.

Hydrocephalus was described by Derry [25] as occurring in an Egyptian (Roman Period) associated with atrophy of the bones of the left side, indicating hemiplegia. Another case of hydrocephalus in remains from a Merovingian cemetery at Weimar has been reported on by Pfeiffer.[26]

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21. Smith, G. E.: Lancet **2**: 521, 1908.

22. Raymond, Paul: Les maladies de nos ancêtres de l'age de la pierre, Aesculape **2**: 122, 1912.

23. Hrdlička, A.: Handbook of American Indians, Washington, Bureau of American Ethnology, Bull. 30, pt. 1, 1907, p. 96.

24. Smith (footnote 6, first reference, p. 38).

25. Derry, D. E.: J. Anat. & Physiol. **47**: 436, 1913.

26. Pfeiffer, L.: Cor.-Bl. d. allg. Aerzt. v. Thüringen **29**: 426, 1900.

Smith [27] described talipes equinovarus in the mummy of Siptah (nineteenth dynasty, about 1410 B.C.). Dawson mentioned another reported by Murray as observed in a mummy of the twelfth dynasty. Ruffer and Dawson copied a number of ancient Egyptian drawings of clubfoot in their articles on dwarfs and deformed persons.[28]

Cretinism was diagnosed in connection with an Egyptian skull of the eighteenth dynasty by Seligmann,[29] who discussed the points of distinction from achondroplasia.

Rickets.—One is impressed with the small amount of evidence of rickets in the material from ancient Egypt. Wood-Jones,[13] in 1908, found none in 6,000 bodies. His statement was confirmed by Smith and Dawson [6] in 1924, after a much larger material was available. Ruffer, in his article on dwarfs and other deformed persons in ancient Egypt, reproduced drawings of bow-legged persons who, he believed, must have had rickets. Klebs [30] alluded to the "bow-legged god Bes and the rachitic child on amulets of the Saitic-Persian period (Berlin Museum: von Oefele)." I have met with one reference to rachitic bones of northern Europe of the neolithic period; this was by Carl Fürst,[5] who mentioned several cases in remains from Denmark and a probable case in remains from Norway. There is little recorded evidence of rickets in the bones of ancient America. Clay figures like hunchbacks were alluded to by Whitney;[14] the same author made no reference to rickets as having been seen in the bones examined by him. Lehmann-Nitsche merely mentioned a few bones as rachitic in his report on arthritis deformans. Hrdlička,[23] whose opportunities for the examination of the bones of American Indians have been enormous, said that "rachitis did not exist in the pre-Columbian Indian." It has been suggested that symmetrical osteoporosis of the cranium described in the following section is a condition allied to rickets.

The condition known as osteitis fibrosa (von Recklinghausen) and that known as osteitis deformans (Paget), now considered by many German pathologists under one heading as osteodystrophia fibrosa, seems to have been almost ignored in the study of ancient osseous remains; further research on such material is needed.

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27. Smith (footnote 6, second reference); The Royal Mummies, Cairo, 1912. From the Service des Antiquités de l'Égypte, Catalogue Générale des Antiquités, Égyptienne J. Musée de Caire, nos. 61051-61100.

28. Ruffer, M. A.: On Dwarfs and Other Deformed Persons, Bull. Soc. Archeol. d'Alexandrie, no. 13; reprinted (footnote 1, second reference). Dawson, W. R.: Dwarfs and Hunchbacks in Ancient Egypt, Ann. M. History **9**: 315, 1927.

29. Seligmann, C. G.: Man **12**: 17, 1912. An interesting outlook on cretinism is that of Finkheimer, Ernst: Die kretinische Entartung nach anthropologischer Methode bearbeitet, Berlin, Julius Springer, 1919; abstr., Arch. f. Anthrop., 1923, vol. 55.

30. Klebs, Arnold: Bull. Johns Hopkins Hosp. **17**: 214, 1917.

Moodie [18] mentioned having seen a case of osteomalacia in the bones of an ancient Peruvian; and MacCurdy [17] contributed another case.

Osteoporosis of the Surface of the Cranium.—A symmetrical osteoporosis on the outer surface of the skull, chiefly of the parietal and frontal bones, giving a startling effect (fig. 1), is often seen in the skulls of American Indians and to a smaller extent in those of other races. Though physical anthropologists are familiar with it, the condition seems to be little known to general pathologists.[31] If it is figured in any of the textbooks of pathology, I have overlooked this fact. The omission is no doubt due to its being extremely rare in the modern white race, if it occurs at all. For that reason, the subject will be considered in some detail.

All of the large collections of Indian bones that I have been able to examine have one or many examples. It seems to occur more abundantly in certain localities than in others not far removed. I have obtained the impression that it was more common in ancient Peru than in any other part of America. Hrdlička [16] found it occurring frequently in skulls from the coastal regions of Peru, but not in those from the mountains. In one lot of Peruvian remains, he encountered osteoporosis in eight of 262 skulls from adults, and in three of sixteen skulls from children. I had the opportunity to examine some 176 skulls from Peru at the Field Museum, Chicago, most of them from adults, and noted four with osteoporosis, counting only those in which the condition was marked. The most typical case occurred in the skull of a child. Another of the four cases (no. 168716) was singular in that it involved most of the skull, including the face, except the parietals. Sylvanus G. Morley and G. D. Williams informed me, in personal communications, that it was exceedingly common among the Mayas of Yucatan, chiefly in young persons. It was also frequent in New Mexico and Arizona. Among fifty-four skulls at the American Museum of Natural History, New York, most of them from the southern part of the United States and from the West Indies, I found one skull showing osteoporosis. It was from Georgia. At the Field Museum, Chicago, I examined fifty-four skulls from Cahokia, Ill., and thirty-three from Cross County, Ark., and found one well marked case from the latter locality. At the Museum of the Buffalo Society of Natural Sciences I examined eighty skulls from

31. I cannot find any reference to it in Pommer: Origin and Diagnostic Significance of Osteoporosis, Arch. f. klin. Chir. **136**: 1, 1925, nor in the referat of the German Pathological Society on osteoporosis, Centralbl. f. allg. Path. u. path. Anat. **35**:258, 1924-1925. Osteoporosis, in general, may, of course, result from many different causes. It is not surprising that metastatic tumors of the cranium occasionally produce appearances somewhat like the symmetrical osteoporosis under discussion. A case was reported by Roman: Beitr. z. path. Anat. u. z. allg. Path. **53**:69, 1912.

Fig. 1.—Symmetrical osteoporosis of the upper surface of the cranium. This seems to have been an active case. The sutures are involved to some extent, which is unusual. The skull is that of an adult or a young adult. The size is slightly reduced in the photograph. The resemblance of the surface to pumice stone may be noted. Figure 2 shows a roentgenogram of the same skull. The specimen is an old one, evidently long buried. It probably belonged to an Indian of the State of New York, but there is no definite record as to its source. It is now in the museum of the University of Buffalo (4367). The inner surface of this skull is nearly smooth and is normal. The skull shows a moderate degree of deformation, doubtless unintentional and common among many tribes of Indians.

western New York and the adjacent part of Ontario and found no case of symmetrical osteoporosis. Figure 1 shows a specimen of uncertain origin, probably from a New York State Indian. It presents one of the most marked cases of osteoporosis that I have seen, and the amount of osteophytic growth is suggestive of tumor formation. Although it is from an adult or a young adult, I should call it an active case. Figure 2 is a roentgenogram of the same skull. (All the allusions that I have made or shall make in this section to skulls of American Indians refer to material that is ancient in the sense that it may be from 100 to 200 years old to 1,000 or 2,000 years old. It is frequently if not usually impossible to give an approximate date to such material.) Adachi [32] gave beautiful illustrations of symmetrical osteoporosis occurring in a Dyak and in an ancient Egyptian. Wood-Jones [13] described what may be the same thing from ancient Egypt, though his description and illustration leave one somewhat in doubt. The condition described by him, he thought, might have been caused by carrying water jars on the head. Another case in an Indonesian was reported on by Bickel,[33] who gave a survey of the literature. T. Wingate Todd, of Western Reserve University, Cleveland, informed me (in a personal communication) that he had seen the condition once (in the skull of a child, not rachitic) in about 600 modern negro skulls studied by him. The skull of a child 8 years old, found in France, apparently of the Gallo-Roman period, was described by Saint-Perier [34] as an example of congenital syphilis; from the description and the illustration, this seems to me to be a case of symmetrical osteoporosis. Parrot [35] long ago gave descriptions of what he called hereditary syphilis of the bones of the skull, which, in some respects, suggest symmetrical osteoporosis. Three specimens, probably presenting the latter condition and not syphilis, coming from Peru, were mentioned by Parrot as examples of syphilis of ancient American origin.

Though symmetrical osteoporosis is most frequent and most marked on the parietal and frontal bones, the occipital bone is often involved. The temporal bone and sphenoids are involved less frequently and the bones of the face very rarely. By the courtesy of A. Hrdlička, of the United States National Museum, Washington, I have been able to examine the bony remains of three young infants from New Mexico, showing marked osteoporosis of the cranium, in which a considerable part of the rest of the skeleton had been preserved. A similar osteoporosis was well marked on the surface of the bodies of

32. Adachi, B.: Die Porosität des Schädeldaches, Ztschr. f. Morph. u. Anthrop. **7:** 373, 1904.

33. Bickel, B.: Ztschr. f. Ethnol. **49:** 102, 1907.

34. Saint-Perier: Bull. et mém. Soc. d'anthrop. de Paris **5:** 31, 1914.

35. Parrot, Jules: Les lésions osseouses de la syphilis héréditaire, Tr. Path. Soc. London **30:** 339, 1879.

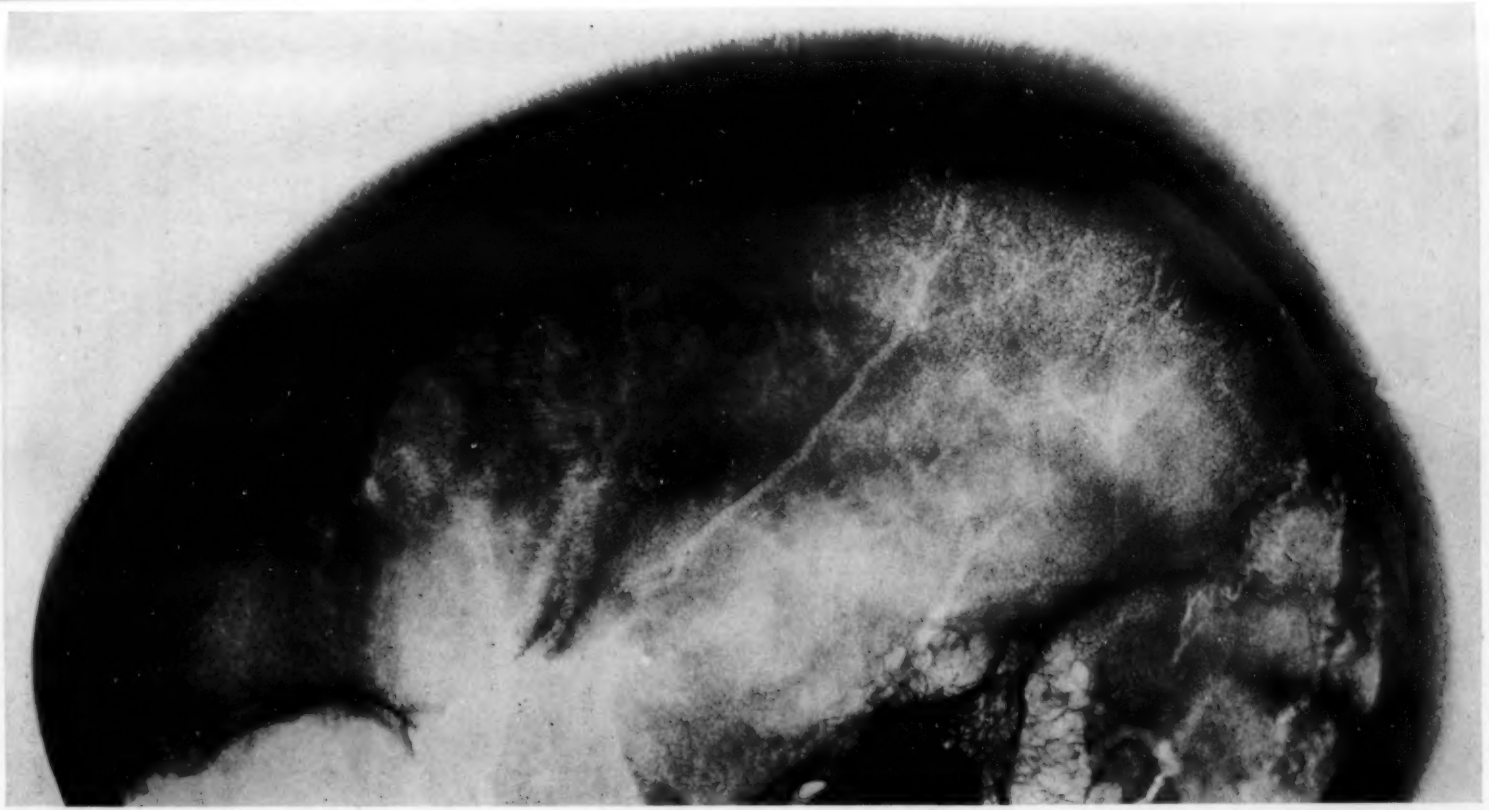

Fig. 2.—The skull with symmetrical osteoporosis shown in figure 1, natural size. A roentgenogram of a skull in the Peabody Museum, Cambridge, Mass., gives practically an identical picture.

the vertebrae, and it appeared also, in traces, externally on the long bones; there was no irregularity of the teeth. MacCurdy [17] told of a case in an ancient Peruvian who at death was probably 70 years old, who had been trephined through an area of osteoporosis of the skull, and who had osteoporosis of the long bones and showed evidences of fractures. The condition is evidently the same as that called cribra parietalia by Adachi.[32] A similar condition seen on the orbital plate (cribra orbitalia) and a somewhat similar condition of the inner surface of the skull (cribra cranii) will be mentioned in an ensuing paragraph. Symmetrical osteoporosis of the ancients was decidedly most common in childhood. The inner surface of the skull is almost never affected. The areas involved, in some cases, are of insignificant size and in others include the greater part of the upper surface of the cranium. Their symmetrical distribution is notable. The sutures are usually avoided. The appearance of the surface has been likened to pumice stone or to coral or to fine sponge or to moss. The outer table may be altogether wanting, while the cancellous bone of the diploë, with enlarged spaces, up to 2 mm. or a little more in diameter, is level with or protrudes a little above the general surface. This lack of depression, or protrusion owing to new growth of bone, distinguishes osteoporosis from the appearance of a portion of skull from which the outer table has been accidentally removed or eroded; there is a slight superficial resemblance between them. In cases like this, the condition is regarded by Hrdlička [16] as active; in other cases, in which the condition may have been arrested or healed, a relatively smooth thickening over the region involved is seen, while the outer table remains perforated with holes from the size of pinpoints up to a diameter of 2 mm. or more. Such an appearance suggested the name of cribrum (sieve). Hrdlička believed that symmetrical osteoporosis began to show itself in infancy; that the underlying condition, whatever it may have been, was frequently fatal; that the process began in the roof of the orbit or on the frontal bone, and extended to other parts of the upper surface of the cranium; and that, in some cases, there was a recovery, but with the relics of the condition often remaining throughout life (fig. 3).

In general, the distribution of the lesions reminds one strongly of that described by some German authors for the lesions of rickets of the skull: symmetrical and avoiding the sutures. These lesions do not, however, occur along the margins of the bones as the lesions in rickets are stated to do. But if I understand correctly, Kaufmann's [36] sketch

36. Kaufmann: Lehrbuch der speziellen pathologischen Anatomie, Berlin and Leipzig, Vereinigung wissenschaftlicher Verleger, 1922, vol. 1, p. 902, fig. 515. Wakeley and Buxton: Surgical Pathology, New York, William Wood & Company, 1929, p. 269.

showing the regions affected by rickets on a child's skull, the areas of involvement are the same as those that one most often sees affected in symmetrical osteoporosis.

The condition that Wood-Jones [37] discussed under the title of "Cranial Ulceration," which was frequent in ancient Nubia, and which he attributed to the carrying of water jars on the head, corresponds, in some respects, to symmetrical osteoporosis; in others, it does not. Apparently, in some of his cases, the soft parts over the skull were preserved, permitting the observation that ulceration had occurred. If symmetrical osteoporosis of the upper surface of Egyptian skulls was mentioned by Smith, Derry, Ruffer or Oetteking, I overlooked the references. Adachi's case has been referred to. Possibly, Wood-Jones worked in a locality where it was unusually frequent, as it was in certain parts of America.

Roentgenologic Observations: The radiating lines, like coarse bristles, replacing the outer table of the skull (fig. 2) are evidently produced by the new bony growths, seen in profile. Two roentgenograms of skulls from Yucatan were sent me by Dr. E. A. Hooton, of Harvard University: one, of a case of acute symmetrical osteo-

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37. Wood-Jones, F.: Archeological Survey of Nubia, Report for 1907-1908, vol. 2, Human Remains, p. 203:

"On carefully examining a skull, it is by no means infrequent to see, either on the vertex or around and above the parietal eminences, some evidences of a more than usually abundant blood-supply to the outer table of the bone. Minute points stand out upon the surface of the parietal bone, and the mouths of small vascular canals are dotted about upon the parietal eminences, and upon the surface of the skull above them. Such a condition is very common among skulls of all periods, and it may be strictly limited to this area of the parietal bones, or it may be much more diffuse: it may be easily overlooked in its earlier stages, and so in some measure its probable, and simple, etiology may be lost sight of. The condition, however, may not be limited to this stage of hyper-vascularity of the surface of the bone: in some cases minute ulcerations of the outer table are seen at the point of maximum intensity of the process; and in other cases this ulceration has spread until it is the dominant feature in the picture.

"At times the process becomes so acute that necrosis of the outer table of the skull has taken place, and portions of the scalp and cranial wall have sloughed away. When this condition is seen only in its advanced stages it is one that naturally suggests some severe disease, and it is not surprising that the syphilitic virus has been invoked as the causative factor yet it is accompanied by none of the other signs of the disease, and a careful study of its etiology shows it to be a process quite distinct from any specific infection. The evidences of cranial periostitis in these cases are usually bilateral, and approximately symmetrical, and the ulceration —when the process is advanced—is at times symmetrical, too. It was noticed from the outset of this investigation that the condition was more common among females than among males, and in Cemetery 23 (for instance) the condition occurred as often as five times in the twelve females bodies."

The author goes on to discuss the possible relation of this condition to the carrying of water jars on the head. The drawing that he gives (his fig. 66), as has been stated, is suggestive of the osteoporosis that is the subject of this section.

porosis, gives a picture nearly identical with that seen in figure 2; the other, of a case of healed symmetrical osteoporosis, gives a picture similar but less distinct. (Roentgenograms of the bones shown in figures 3 and 4 give no more than suggestions of these lines.) Cooley, Witwer and Lee [37a] described similar changes in the skull in certain anemias of childhood. Dr. M. C. Sosman, of the Peter Bent Brigham Hospital, Boston, found a picture like this in the skulls of negroes with

Fig. 3.—Symmetrical osteoporosis of the upper surface of the skull, a healed case, from the Chicama Valley, Peru (after Hrdlička: Smithsonian Collections **61**:57, 1914).

sickle-cell anemia and of white men, usually Greeks or Italians, with von Jaksch anemia (personal communication). I have seen roentgenograms of two cases of metastatic tumor of the cranium that gave radiating lines somewhat like those described.

37a. Cooley, Witwer and Lee: Anemia in Children with Splenomegaly and Peculiar Changes in Bones: Report of Cases, Am. J. Dis. Child. **34**:347 (Sept.) 1927.

Histologic Changes in Symmetrical Osteoporosis: Through the courtesy of A. Hrdlička, I have been enabled to make sections from two moderately well marked cases of osteoporosis in a parietal and a frontal bone of young children, Indians of Arizona and Utah (figs. 4 and 5). The marrow being gone, one could study only the framework of the decalcified bone. The spaces of the cancellous bone of the diploë were large. They opened widely on the surface, and gave the impression that the marrow protruded under the periosteum. These openings were evidently the equivalent of those present on the normal skull for the passage of vessels, well developed on the parietal bones of normal infants

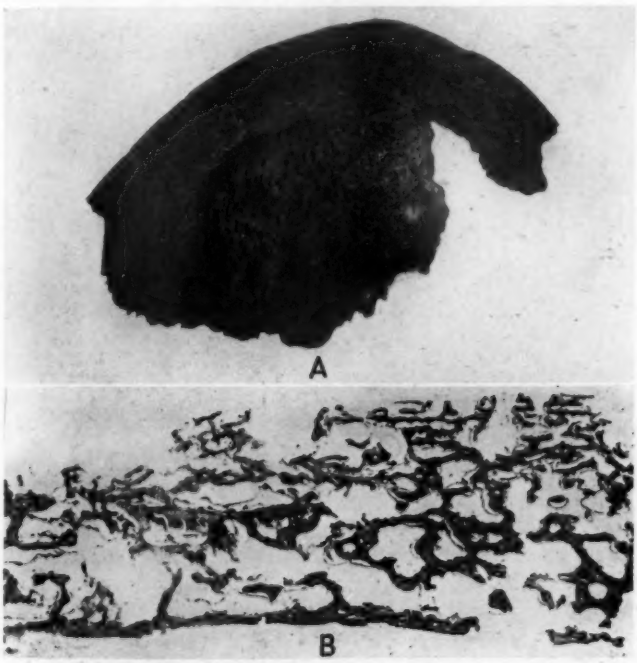

Fig. 4.—*A* represents symmetrical osteoporosis of the frontal bone of a child, an active case, from Utah; *B*, a very low power magnification of a section from the bone shown in *A*. The osteophytic growth on the outer table may be noted.

but of exaggerated size in symmetrical osteoporosis. In one of the skulls, in which the disease was shown still acute and progressive (fig. 4 *A*), the outer table could barely be traced through the area involved, and new cancellous bone projected above the general surface. The impression obtained from these sections was that they had much similarity to the periosteal formation of osteoid tissue and bone seen in rickets.[38] Sec-

38. One of the illustrations of sections of the skull from cases of rickets in Shattock's article on "Morbid Thickening of the Calvaria," etc. (Tr. XVIIth Internat. Cong. Med. London, 1913, sec. 3, Pathology, p. 3) is strikingly like the picture from one of my sections (fig. 4 *B*).

tions were made from the ribs of two other ancient Indians of New Mexico, both having marked osteoporosis of the parietal bones. The large size of the spaces of the cancellous bone of these ribs was striking (fig. 6). There were points where the rib was little more than a shell that once contained marrow. The junction of the rib with the cartilage was not like that seen in rickets. These changes were suggestive of some systemic disorder affecting the bone marrow in general. I do not know of any studies on the histologic changes in which the

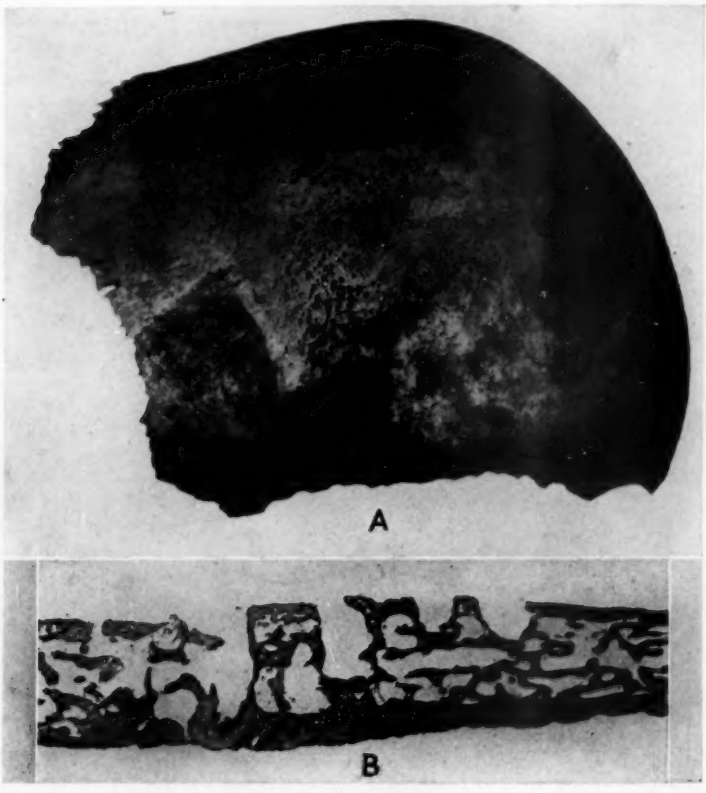

Fig. 5.—*A* represents symmetrical osteoporosis of the parietal bone of a child from Arizona, a practically healed case; *B*, a low power magnification of a section from the bone shown in *A*. The openings are in the outer table.

marrow and the periosteum were found preserved. It is possible that such material could be obtained from autopsies on modern Indians.

Von Hansemann,[39] in his monograph on rachitis of the skull, considering chiefly conditions found in apes kept in captivity, described and

39. Von Hansemann, David: Die Rachitis des Schädels, Berlin, A. Hirschwald, 1901.

illustrated some conditions that are like the symmetrical osteoporosis of human skulls, while other conditions that he described, I should think, were more like osteitis deformans (Paget's disease) or leontiasis ossea. The tendency of the condition shown in his apes to spread to the bones of the face is not exhibited in the symmetrical osteoporosis under discussion, which, as Hrdlička [16] pointed out, rarely appears on the face. T. Wingate Todd informed me (personal communication) that he found symmetrical osteoporosis in the skull of a monkey, *Lagothrix lagotrica*, that had calcium deficiency but not rickets.

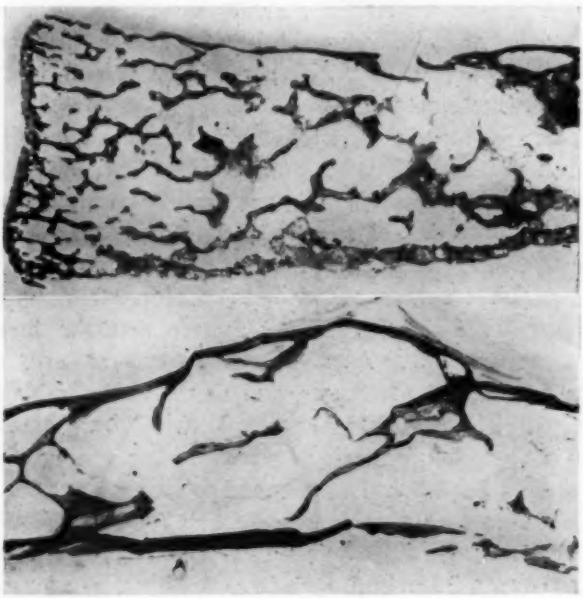

Fig. 6.—Very low power magnification of sections near the ends of the ribs of two skeletons that showed marked symmetrical osteoporosis of the skull. It may be noted that the costochondral junction, shown in the upper section, is not like that of rickets. The skeletons were those of Pueblo Indian children of New Mexico.

Practically no information exists as to the causation of symmetrical osteoporosis. Irritation and pressure have been mentioned as possible causes. The influence of toxic disorders (Hrdlička), endocrine disorders, disturbances of calcium metabolism, lack of some vitamin and other dietary deficiencies suggest themselves as possibilities. The food of the Indians and of the poor people among ancient Egyptians viewed from the modern standpoint was miserable and the supply precarious. As for the sedentary Indians, both of North and of South America, maize or Indian corn was the most important item in their food and it

may be worthy of note that maize is said to be deficient in the antiscorbutic vitamin C and probably in the antirachitic vitamin D. On looking at Wissler's [40] map of the food areas of the New World, it is seen that his "area of intensive agriculture" corresponds closely with the regions from which most of the cases of symmetrical osteoporosis have been reported. However, rickets, as one now sees it, seems to have been infrequent among the Indians. There was certainly an abundance of sunlight available in most of this area. The infant's body, however, would be little exposed if it was constantly wrapped up. The examinations which I have given are the only ones that I know of for the eastern half of the United States; they indicate that symmetrical osteoporosis occurred there but was not common. In this area, agriculture was practiced perhaps less intensively, and here, again, maize was the staple product, for the most part.

The results of roentgen examinations of the skull in anemias of children are most suggestive. It is to be hoped that the relation of the condition described in these anemias with symmetrical osteoporosis may be determined by further study. Tello [41] discovered a Peruvian skull, now in the Warren Museum, Harvard Medical School, Boston, in which trephining had been performed through an area of osteoporosis, which, of course, suggests that the operation had been done to relieve pain. As mentioned later, MacCurdy and Muniz and McGee reported similar cases, and there is another in the United States National Museum, Washington, D.C. (266064 Peru). It has occurred to me that the pressure produced on the occiput of the Indian baby resting on a head board would tend to create a venous hyperemia in the parietal and frontal regions, the venous outlets for the posterior region being impeded. Such hyperemia might cause a localization of osteoporosis over the parietal and frontal bones, not operating as a cause by itself but in combination with some of the causes enumerated. In cases of intentional deformation, pressure was made also on the frontal region, and sometimes about the sides of the head, so that one would expect venous hyperemia of the vertex to have been even more marked. The use by many Indians of a "tump-line" passing over the forehead to carry heavy loads must have exerted strong pressure on the frontal region, and might be considered as a factor in young persons, but I should not lay much stress on the tump-line.

Cribra orbitalia is the name given by Welcker [42] to a condition much like cribra parietalia (symmetrical osteoporosis) but located on the

40. Wissler, Clark: The American Indian, ed. 2, New York, Oxford University Press, 1922, p. 2.

41. Tello, J. C.: Prehistoric Trephining Among the Yauvos of Peru, Proc. Internat. Cong. Americanists, London, 1913, pt. 1, p. 75.

42. Welcker: Cribra orbitalia, Arch. f. Anthrop. **17**: 1, 1888.

upper wall of the orbit and usually covering a much smaller area. It is found often in children and has been described as occurring in Egyptians, ancient and modern, in Japanese, in Europeans and in Negroes and is seen in the skulls of American Indians. According to Toldt, quoted by Oetteking,[43] there is an increased formation of spongy bone, the outer table is but slightly developed, there is a change in the circulation so that blood flows through the periosteal veins rather than through those of the diploë, and a spongy new growth of bone of the nature of osteophytes is formed; otherwise the orbital plate is thin and without diploë.

Cribra cranii (Waldeyer) is a condition affecting the inner surface of the skull, described at length by Koganei,[44] who regarded it as related to cribra orbitalia, but was uncertain as to cribra parietalia. His cribra cranii is frequent in children, and it occurs in modern Germans and Japanese, as well as in ancient peoples. It is commonest on the inner surface of the frontal bone, and seems related to the impressions made by the blood vessels. There is an abundance of marrow in the spongy bone, and in recent cases the formation of new layers of bone can be recognized. He regarded it as similar to the osteophytes of the puerperium.

The descriptions and illustrations of the histologic changes in cribra orbitalia and cribra cranii are like the pictures that I obtained in symmetrical osteoporosis.

Fractures and Injuries.—Whether or not the conditions under which men lived in earlier times were more conducive to bodily injuries than those of modern civilization might be an interesting if not a profitable subject for speculation. It is certain that every large collection of ancient or prehistoric bones has numerous examples of fractures, healed or otherwise.

Perhaps as celebrated as any fossils that have ever been found are the bones of Pithecanthropus from Java, usually ascribed to the late tertiary period, therefore antedating the glacial period. If not the bones of a man or of a direct ancestor of man, they are at least those of a cousin of the ancestor of man. The left femur presents an enormous, irregular exostosis on the inner side of the upper third; evidence of fracture is not present. I have seen only casts and photographs of this femur, but the abnormal and redundant new growth of bone is so like that occurring about many healed fractures that I should suppose it had been caused by injury. Dubois,[45] the discoverer of the specimen,

43. Oetteking: Kraniologische Studien in Altegyptern, Arch. f. Anthrop. **36:** 49, 1909.

44. Koganei, Y.: Cribra cranii und Cribra orbitalia, Mitt. a. d. med. Fak. d. k. Univ. zu Tokio **10:** 113, 1913.

45. Dubois, E.: Ueber die Hauptmerkmale der Femur von Pithecanthropus erectus, Anthrop. Anz. **4:** 131, 1927.

described marks at various points about the upper end of the femur that he ascribed to the teeth of a crocodile, which I interpret as giving support to the supposition that the exostosis was caused by injury.

The Neandertal race belonged to the latter part of the glacial period, and the famous but incomplete skeleton from which this race was named is now in the Provincial Museum, Bonn. The left humerus and ulna also show deformity probably caused by injury.[46] Among the bones of an early type of Neandertal man found at Krapina, Croatia, there is a clavicle with a well united fracture.[47] The Rhodesian skull from South Africa, of undetermined but ancient age and of extraordinarily brutal type, resembles the Neandertal skulls in many respects. It shows evidence of a severe injury above one ear, which was probably followed by suppuration.[48]

One of the two skeletons found at Obercassel in Germany, referred to the late paleolithic (Magdalenian) period, bears the marks of an old injury on the left parietal bone and of an old united fracture of the right ulna.[49] One of the group of skeletons discovered at Cro-Magnon (Aurignacian) bears the plain marks of a blow on the forehead. These bones belong to the early part of the period of the cave artists, which is roughly around from 20,000 to 30,000 years ago.

There is nothing surprising in these facts unless it is that the evidence of injury should have been preserved from such remote times.

Wood-Jones [13] described a great number and variety of fractures found in the bones of the ancient Nubians. He made interesting comparisons between the incidence of these fractures in the various parts of the body and the incidence of fractures in the corresponding parts of the body in the great cities of the present day. He found the results of whatever treatment was used good, and was especially amazed at the evidences of recovery from extensive fractures of the skull. He remarked that sepsis rarely followed even severe fractures that must have been compound. He gave sketches that adequately illustrate the distribution and the types of the fractures.

MacCurdy [50] called attention to the large number of fractures of the skull among the ancient Peruvians, which he attributed to the impacts of the war clubs used in their hand-to-hand combats.

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46. Hrdlička, A.: The Most Ancient Skeletal Remains of Man, Rep. Smithsonian Inst. for 1913, Washington, 1914, p. 520.

47. Gorjanovic-Kramberger, Karl: Der Diluviale Mensch von Krapina in Kroatien, Wiesbaden, Kreidel, 1906.

48. Keith, Arthur: The Antiquity of Man, ed. 2, London, Williams & Norgate, 1925, p. 417.

49. Verworn; Bonnet, and Steinmann: Der diluviale Menschenbefund von Obercassel bei Bonn, Wiesbaden, 1919.

50. MacCurdy, G. G. (footnote 17, p. 236).

Many museums have bones of the neolithic and later periods with flint or bronze arrow or spear points still embedded where they lodged. I have seen numerous specimens of this kind among bones of American Indians. Such relics have a certain dramatic quality, but they do not give any new information as to the pathologic changes in bone. The results of injuries of bone in ancient times do not seem in any way different from those that are observed today. Cases of ankylosis and of dislocation have been found many times among prehistoric bones, differing in no respect from those that one sees at the present time.

Elliott Smith [51] described and illustrated with excellent plates a fracture of the femur and one of the forearm that were enclosed in splints in a skeleton from tombs of the fifth dynasty in Egypt; that is, from 4,000 to 5,000 years ago. Another and later example was recorded by Wood-Jones.[13]

Moodie [52] described and gave good illustrations of a well preserved bandage remaining on an ancient head from Peru, consisting of cotton cloth placed over the occiput and held in position by woolen cord.

Wood-Jones [53] reported on an examination of the bodies of 100 men executed in Nubia in Roman times, which exhibited unusual results, apparently from hanging, which are of interest to students of medicolegal problems; and in his works on the Archeological Survey of Nubia, he cited many other examples of injuries.

Primitive and Prehistoric Trephining.[54]—Trephined skulls from neolithic graves in France were found some time before their nature was understood. The story of the manner in which they came to be correctly interpreted is of some interest; I have condensed it from MacCurdy.[55]

The pioneer archeologist of America, E. G. Squier, while in Peru, obtained an ancient Inca skull that had been trephined by making two pairs of parallel incisions in the skull at right angles to each other. Squier submitted this skull [56] to Broca, who recognized it as an example of trephining; later, Broca realized from this specimen the true nature of the European specimens. Prunieres about the same time (1870-

51. Smith, G. E.: The Most Ancient Splints, Brit. M. J. **1**:732, 1908.

52. Moodie: Ann. M. History **8**: 69, 1926.

53. Wood-Jones: Brit. M. J. **1**: 736, 1908.

54. This subject has been discussed in many articles, only a few of which can be mentioned here. The citations that I am making give many references to the literature.

55. MacCurdy (footnote 8, first reference, vol 2, p. 165; footnote 17, p. 236). These contributions include good plates.

56. According to MacCurdy, this skull is now in the American Museum of Natural History, New York. A good photograph of it appeared in Muniz and McGee: Primitive Trephining in Peru, Rep. Bur. Am. Ethnol., Washington **16**: 11, 1894-1895.

1880) described trephining in neolithic skulls. Broca used a paleolithic chipped flint to trephine the skull of a 2 months old dog; it was done by the scraping process and required eight minutes; the wound healed promptly. The two methods just mentioned appear to have been those most commonly used; rarely cauterization may have been employed, or possibly, as suggested by Lucas-Championnière, a series of perforations close together and in the form of a circle, to permit the removal of a round plate of bone.[57]

MacCurdy gave a photograph of a Peruvian skull (fig. 7) which he thought held the record, and probably with justice; for it had been successfully trephined five times. Many cases of trephining are found in which healing had not taken place, the patient succumbing to the operation or to the condition for which the operation was performed. The openings that do not show signs of healing may have been made post mortem as part of some ritual or to secure bone amulets.

Muniz and McGee [56] thought that the surgery in Peru "was crude in plan and bungling in procedure." They admired the vitality of the patient, rather than the technic of the operator. They believed that the mortality must have been high. (Their monograph includes good plates, some of them showing the removal of a rectangular plate of bone by two pairs of parallel grooves at right angles to each other.) MacCurdy, on the other hand, was impressed with the skill of the Peruvian surgeons, and Moodie said that healing often seemed to have been rapid.

Evidence of prolonged suppuration following the operation is not common. Proof of the method by which the operation was performed is not usually preserved in the old bones. In the skulls that have healed, the edges of the openings are usually round or beveled.

The frontal and parietal bones were the parts usually operated on. The forehead low down, and the occipital and the temporal bones were only rarely attacked. A healed injury or a large persistent parietal foramen may look somewhat like an example of trephining.

Several different purposes have been supposed to have animated the operators, who were doubtless the medicine men of the tribe or their equivalent. First, the object may have been to permit the escape of evil spirits from the skull in cases of epilepsy or insanity. Second, the operation may have been done to cure local disease or in the treatment of fractures, or third for the relief of headaches. A few skulls with symmetrical osteoporosis that had been trephined have been found in Peru. Tello,[41] MacCurdy [17] and Muniz and McGee [56] gave illustrations of this, and there is a skull showing it in the United States National

57. A good photograph of a skull from Peru in which this method had been tried is given in Moodie, R. L.: Ann. M. History **9**:277 (his fig. 19 B), 1927.

Fig. 7.—A skull that has been successfully trephined five times. It is that of an adult male of Patallacta, Peru (after MacCurdy, G. G.: Am. J. Phys. Anthrop. 6: 265, 1923).

Museum, Washington (266064). MacCurdy [17] believed that he had seen clear evidence that in Peru trephining was frequently done for the relief of fractures probably the result of combats, and perhaps in the treatment for disease. Tello [41] offered substantially the same opinion. Moodie [57] stressed the use of the sling by the old Peruvians, as frequently causing fractures of the skull, and the attempts to relieve depressed fractures by trephining. Moodie [58] quoted Hrdlička to the effect that trephining was done for surgical reasons, also to the effect that the opening was sometimes covered with a plate made of gourd or of shell or of silver. At the meeting of the International Congress of Americanists in New York, September, 1928, Julio C. Tello, curator of the Peruvian Archeological Museum, Lima, exhibited two remarkable skulls; in one of these, a thin polygonal plate of beaten gold had been laid over the circular hole in the skull; apparently healing had taken place with this plate in position, although it was not possible to be certain as to this point, for the specimen was shown as it came from the ground, without cleaning. The other skull was that of a subject who must have died shortly after the operation; a suture of cotton string was still present in the scalp over the opening in the skull. Posansky,[59] from his observations in the highlands of South America, stated that the present-day Indians perform severe operations after having stupefied the subject with a native brew of alcohol and by the local application of coca leaves. He thought that trephining was performed under similar conditions. The use of coca leaves has been suggested by others; but, as strong solutions of cocaine are said not to have any anesthetic influence on the unbroken skin, the advantages obtainable from coca leaves used locally must have been limited. When the scalp was lacerated, as in compound fractures, the patient must often have been rendered comatose by the injury.

Prehistoric trephining was probably carried out more frequently in Peru than in any other part of the world. Muniz and McGee [56] found nineteen cases of it in about 1,000 skulls, while MacCurdy [17] reported that in a series of 250 craniums, 18 per cent had been trephined. Bandelier [60] found sixty-five skulls trephined (by scraping) in about 1,200 from Bolivia. According to Bandelier, trephining was still practiced in Bolivia in 1904, though rarely and secretly. Among 176 skulls from Peru at the Field Museum, Chicago, I found two examples of trephining, one fresh and one healed.

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58. Moodie (footnote 52, p. 397).

59. Posansky, A.: Ueber Trepanieren und künstliche Verunstaltungen an Aymara Schädeln, Arch. f. Anthrop. **56**: 158, 1924.

60. Bandelier, A.: Aboriginal Trephining in Bolivia, Am. Anthrop. N. S. **6**: 440, 1904.

Trephined skulls from the Tarahumare Indians of northern Mexico have been reported.[61] Two trephined skulls found in New Mexico were described by Shapiro.[62] The United States National Museum, Washington, has one from Michigan (147272). Greenman[63] mentioned examples from Michigan. Smith[64] reported on such skulls from Canada. Others may have been described, but these are sufficient to show that while examples are found in North America, they are rare.

Numerous trephined skulls have been found in France, Switzerland, Bohemia, Moravia and other parts of Europe, chiefly western Europe, including Denmark and Sweden (Fürst[5]) and the Canary Islands. Parry[65] was able to learn only of three found in England, and one of these was doubtful. Most of the European specimens belong to the neolithic period; a few are said to be of the bronze age and even of the iron age.[66] The practice may have continued down to historic times.

A somewhat doubtful specimen from Egypt, 200 A.D., was reported by Ruffer,[67] and another was reported by Derry,[68] who, however, was of the opinion that the opening was either congenital or, more probably, due to a dermoid cyst. The absence of trephined skulls from Egypt is noteworthy when one realizes that many thousand Egyptian skulls have been studied by competent observers.

Ruffer gave a valuable review of the knowledge on this subject, including trephining as now practiced by the Kabyles of North Africa and other modern tribes.[69]

Cranial Scars.—A rare lesion of neolithic skulls from dolmens in France was described by Manouvrier.[70] It consists of some erosion and

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61. Lumholtz and Hrdlička, A.: Am. Anthrop. **10**:389, 1897.

62. Shapiro: J. Am. Mus. Nat. Hist. **27**: 266, 1927.

63. Greenman: Am. Anthrop. N. S. **28**:312, 1926.

64. Smith, H. J.: Am. J. Phys. Anthrop. **7**:447, 1924.

65. Parry: Lancet **1**:1699, 1914.

66. MacCurdy (footnote 8, vol. 2, p. 405). Matiegka, J.: La trépanation et autres opérations sur la tête a l'epoque préhistorique sur la territoire de la Tchécoslavique, Anthropologie, Prague **6**:41, 1928. Sudhoff, Karl: Medizin in der Steinzeit, Ztschr. r. ärztl. Fortbild. **6**:196, 1909.

67. Ruffer (footnote 1, second reference, p. 196); Some Recent Researches on Prehistoric Trephining, J. Path. & Bact. **22**: 90, 1918.

68. Derry, D. E.: J. Anat. & Physiol. **48**:417, 1914.

69. Recent studies giving methods of trephining now used by savage tribes are: Sarasin, F.: Abstr., Anthropologie **29**:151, 1918-1919. Wölfel, D. J.: Die Trepanation: Studien über Ursprung, Zusammenhänge und Kulturelle Zugehorigkeit d. Trepanation, Anthropos., 1925, vol. 20; brief review by MacCurdy: Am. Anthrop. **29**:118, 1927.

70. Manouvrier, quoted by MacCurdy, G. G.: Prehistory Surgery, A Neolithic Survival, Am. Anthrop. N.S. **7**: 17, 1905; also mentioned in MacCurdy (footnote 8, vol. 2, p. 166). A good photograph of the lesion is shown in the article on prehistoric surgery.

some new formation of bone on the upper surface of the skull in the form of a line beginning just above the anterior curve of the frontal bone, extending to the posterior part of the parietal bones, where it runs into a lateral branch making a kind of T (sincipital T). The scars may be deep enough to produce a form of trephining. The scars are believed to have been made by lesions of the scalp, probably cauterization, deep enough to have affected the periosteum. Quotations that are given from Avicenna and Albaucasis indicate that such cauterization was employed in their day in the treatment for epilepsy and melancholia. It is suggested that a form of treatment used in neolithic times survived and was still practiced in the middle ages.

Somewhat similar oval scars in the region of the anterior fontanel have been described as occurring on the crania of the Guanches, ancient inhabitants of the Canary Islands; rarely these give evidence of grave suppuration; obviously, they were produced by some form of injury. Historical accounts indicate that scarification for the relief of pain was used by the Guanches.[71]

A skull from Peru that may be an example of some kind of scarification was described by Moodie.[72] MacCurdy [17] saw what he considered a somewhat doubtful case of cauterization in a skull from Peru.

Osteomyelitis, Osteitis and Periostitis.—Practically all reports on the diseases shown in ancient bones refer to the bone changes known under one or all three of these names. This statement applies to neolithic Europe, to Egypt and to North and South America. It is not always easy to distinguish between postmortem erosion of old bones by natural causes and destruction produced by inflammation of bone. The difficulty of determining, without clinical history or other aids, whether the changes in an isolated dried bone were produced by suppurative osteomyelitis or by tuberculosis is evident to any pathologist. Wood-Jones [13] remarked on the rarity of evidence of infection following even severe fractures. Remembering the numerous specimens showing destructive inflammation of bone in museums of modern pathology, I have, on the whole, been impressed with the rather small amount of material of the same kind that has come down from ancient times. An exception, however, may be made of the alveolar processes of the jaws, which will be considered in connection with the subject of ancient teeth. Wood-Jones reported a few cases of destructive disease of bone, among

71. Lehmann-Nitsche, Robert: Lésions de crânes des Iles Canaries, Rev. del Museo de la Plata **11**: 211, 1903. This article gives references to the literature, and discusses the lesions on the skulls of the Guanches, and describes the method of scarification in detail. Bockheimer, Bregmanarben und ihre mutmassliche Entstehung nach Untersuchungen an Guancheschädeln, Arch. f. Anthrop. **54**: 131, 1922. Bockheimer's references constitute a review of the literature.

72. Moodie, R. L.: Am. J. Phys. Anthrop. **4**: 219, 1921.

them one of perforation of the hard palate due to chronic rhinitis. His remarks on osteitis of the upper surface of the cranium are referred to in the section on symmetrical osteoporosis. Smith and Dawson [6] remarked that mastoid disease was frequent in ancient Egypt and Nubia; it is occasionally seen in Indian bones from North and South America. Irregular thickening of the long bones, especially the tibia, has been found common in ancient Indian remains both from North and South America, and has been described by many investigators. The possibility that the condition may have been caused by syphilis was brought forward long ago and has occasioned much controversy, with indecisive results.

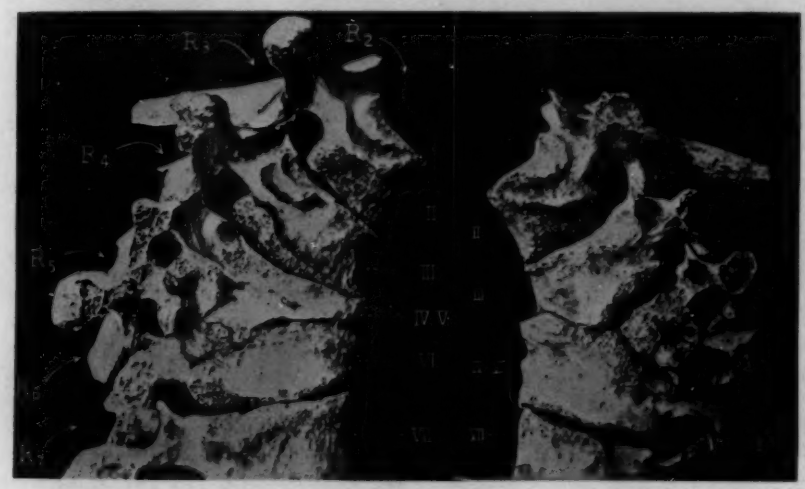

Fig. 8.—Dorsal vertebrae of a neolithic skeleton found near Heidelberg, Germany, described by Bartels (Arch. f. Anthrop. **6**: 243, 1907) as an example of Pott's disease. Reproduced by permission of the editor of the Archiv für Anthropologie.

Syphilis.—It is my intention to consider the subject of prehistoric syphilitic bone disease in a separate paper, which is now in preparation. It will be sufficient to say that at the present moment I cannot learn of any published case that is known to antedate the year 1500 A.D. and that can confidently be pronounced syphilis of bone.

Tuberculosis.—The evidence for the existence of tuberculosis in ancient times is derived from a small number of cases, and much of this evidence is not convincing. A neolithic skeleton (fig. 8) found near Heidelberg has been thoroughly described by Bartels,[73] and has been

73. Bartels, Paul: Tuberkulose (Wirbelkaries) in der jüngeren Steinzeit, Arch. f. Anthrop. N.F. **6**: 243, 1907.

referred to by several writers. The specimens came from a young adult. The bodies of the fourth and fifth dorsal vertebrae were almost entirely missing; the remnants of them were fused with the body of the sixth. As a result, there was kyphosis and some scoliosis to the right; narrowing of the spinal canal was not observed. Bartels believed that arthritis deformans might be excluded as an element in the case, and also fracture. The illustrations seemed to me not entirely convincing in excluding impacted fracture. Raymond [22] alluded to the frequency of tuberculosis in bones of the stone age, but did not tell of any new material, except a specimen showing what probably is spinal disease of the bronze age, from Nimes, said to have been described by Poncet and Leriche. Fürst [5] stated that the earliest known example of tuberculosis in Denmark is one of spondylitis in a child of the twelfth century. In Egyptian remains, there is considerable evidence for tuberculosis, which was set forth by Smith and Dawson,[74] Derry and Smith [75] and Smith and Ruffer.[76] The material consists of a case of disease of the hip joint in a child from the fifth dynasty, about 2700 B.C., and eight cases considered to be tuberculosis of the spine from ancient Nubia. One of these from the middle Nubian period, which I should judge to be around 2000 B.C., was described by Derry [77] as follows:

> A young woman about 21 years of age, whose lumbar vertebrae showed the following pathologic conditions: The first three vertebrae of the series were involved in the disease, which consisted in an ulceration of the centra of the vertebrae. The inflammatory process had completely destroyed the body of the second lumbar vertebra, which was firmly ankylosed to the first vertebra. The latter, owing to the loss of support from below, had fallen forward, so that its upper surface faced anteriorly, and it rested upon the surface of the third. The centrum of the latter was much eaten away by the disease, and formed, with the superimposed centrum of the first lumbar and the pedicles and laminae of the second, a large abscess cavity. When these three diseased vertebrae were placed in position with the other lumbar and lower dorsal vertebrae, a most striking picture of acute curvature incident to Pott's disease was revealed. No other signs of tubercular disease were met with in the rest of the skeleton.

Four other convincing cases of the Nubian archaic period are described by Derry.[78] Finally, there is the mummy (figs. 9 and 10) described in detail by Smith and Ruffer,[76] that of a priest of Amen of

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74. Smith and Dawson (footnote 6, second reference).

75. Derry, D. E., and Smith, G. E.: Bull. Archeol. Survey of Nubia, 1909, no. 6, p. 29.

76. Smith, G. E., and Ruffer, M. A.: Pottsche Krankheit an einer aegyptischen Mumie aus der Zeit der 21. Dynastie (um 1,000 V. Chr.), in Sudhoff and Sticker: Zur historischen Biologie der Krankheitserreger, Giessen, 1910, no. 3; reprinted in Ruffer (footnote 1, second reference).

77. Derry, D. E.: Bull. Archeol. Survey of Nubia, 1908, no. 3, p. 31.

78. Derry, D. E.: Bull. Archeol. Survey of Nubia, 1910, no. 5, p. 21.

the twenty-first dynasty, about 1000 B.C. The specimen presented a marked angular curvature, projecting posteriorly, in the region of the eighth and ninth dorsal vertebrae. Extensive destruction of the last three or four dorsal vertebrae and of the first lumbar vertebra was evident. There had been some new formation of bone at the first lumbar vertebra. A swelling on the right, beginning at the last lumbar

Fig. 9.—A probable case of Pott's disease of the spine in the mummy of a priest of Amen, dating from the twenty-first dynasty in Egypt (from 1090 to 945 B.C., according to Breasted). Figures 9 and 10 are reproduced by permission of G. Elliot Smith, who took the photograph, and for whom the drawing, figure 10, was made by Mrs. Cecil Firth.

vertebra, was traced down into the iliac fossa, without disclosure of any opening through the skin; it was regarded as a psoas abscess. This view seemed justified on account of the destruction in the region of the

right psoas muscle as compared with the left, although pus and cocci or bacilli could not be demonstrated (only molds). Trachea, larynx and bronchial lymph nodes were examined in sections for tubercle bacilli, and none were found. Although it is not stated that a search was made for tubercle bacilli in tissue from the psoas abscess, presumably such a search was made. This specimen seems as convincing as any ancient material could be expected to be, unless tubercle bacilli could be shown

Fig. 10.—Drawing of the priest of Amen whose mummy, showing what is probably Pott's disease, is seen in figure 9.

to be present and this is not always easy to do in fresh cases of Pott's disease. Wood-Jones mentioned a lung of the Byzantine period thought to be tuberculous, though tubercle bacilli could not be found. Ruffer [79] reproduced figures from ancient Egypt that he believed were intended to represent the humpback of Pott's disease.

79. Ruffer (footnote 28, first reference).

Although a number of reports on diseases of ancient bones of various parts of North and South America have been contributed, not many allusions have been made to anything that might be tuberculosis. Whitney,[14] who was a competent pathologist, described, without making a diagnosis, a case of destruction and ankylosis of the lower cervical and upper dorsal vertebrae from stone graves of Tennessee, and clay images representing hunchbacks. Means [80] mentioned what may have been a case of Pott's disease in mound builder remains from Ohio. Hrdlička [81] stated that "as yet no bones of undoubted pre-Columbian origin have been found that show tuberculous lesions, and such lesions are very rare in Indian bones dating from the period of the earliest contact with the whites." [82]

The excavations conducted by Kidder, of Andover, Mass., at Pecos, N.M., have yielded a large amount of ancient osseous remains of the Pueblo Indians. Several specimens exhibiting what is thought to be tuberculosis of the vertebrae were found. Through the kindness of Hooton of the Peabody Museum, Cambridge, Mass., I have been able to give some of these specimens a brief examination, and they seemed to me to be promising. As has been stated, a report on the osseous material by Hooton,[19] to be issued by Phillips Academy, Andover, Mass., is in preparation.

Arthritis Deformans.—New formation of bone about the joints and the accompanying deformities and ankyloses are evidences of disease that lend themselves well to preservation. I shall make no attempt to consider the numerous synonyms for and classifications of chronic proliferative joint disease of unknown etiology, but shall consider the conditions thus named collectively under the designation arthritis deformans. This disease, or something much like it, has been observed many times in various fossil vertebrates, as it has been also in wild and domestic animals. In the cave bear, it was common enough to have been endowed with the name, "cave gout" (goutte de cavernes, Höhlengicht).[83] Its occurrence in ancient skeletons of man is consistent with these facts.

Certain interesting but fragmentary skeletal remains found at Krapina, Croatia, have been referred to the Neandertal or Mousterian race. They seem to belong to the last interglacial period (Riss-Würm), and in that case are early Neandertal. Some of the bones from Krapina, notably one lower jaw, show evidences of arthritis, which is called

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80. Means, H. J.: Am. J. Roentgenol. **13**: 359, 1925.

81. Hrdlička, A.: Bull. Bur. Am. Ethnol. Washington, 1909, no. 42, p. 1.

82. The early history of tuberculosis among the Indians is summarized in "Tuberculosis among the North American Indians," Report of a Committee of the National Tuberculosis Association, Senate Committee Print, Washington, 1923.

83. Virchow, R.: Dermat. Ztschr. **3**: 4, 1896.

arthritis deformans. The descriptions and plates [84] suggest that the process at the articulation of the mandible may have been an infectious arthritis, since a fistula is described as occurring below the joint and apparently pyorrhea, also; an ulna, two patellas and some vertebrae apparently show arthritis deformans. Keith [48] stated that there is some "rheumatic change" in the form of lipping of the left tibia in the Rhodesian skeleton, which is of undetermined but probably ancient age, and belonged to a relative of the Neandertalers. The Neandertal skeletons are from 30,000 to 50,000, or more, years old.

At the end of the glacial period in the reindeer period, in which men of modern skeletal type began to appear, there were also examples of arthritis deformans (from 15,000 to 30,000 years ago).

At Lyons, France, in 1925, Dr. Lucien Mayet showed me an example of spinal arthritis deformans in a skeleton from Solutré, provisionally assigned to an Aurignacian level. One of the Obercassel skeletons, believed to be Magdalenian, is described as having general arthritis deformans (arthritis ossificans).[85] The skeleton from Chancelade, in central France, excavated from a lower Magdalenian level, gives marked evidences of arthritis deformans (osteo-arthritis) about the head of the right humerus and scapula, according to the description of Testut, quoted by Ruffer.[86]

Arthritis deformans was frequent in western Europe in the neolithic age and bronze age and in Roman times.[87]

Egypt has furnished more examples of arthritis deformans [88] from ancient times than any other locality, and the recognition of this fact is owed to the admirable work of Wood-Jones. From his investigations and from others, it seems that arthritis deformans was common in Egypt and Nubia, even in the archaic and predynastic periods, 4000 B.C. or earlier. Ancient bones showing it date also from various dynasties, and from the occupation by the Greeks and the Romans, and from the time of the Copts, about 500 A.D. The disease is also common

84. Gorjanović-Kramberger, Karl (footnote 47); Umschau **12**: 623, 1908.

85. Verworn, Bonnet and Steinmann (footnote 49, pp. 10 and 192).

86. Ruffer (footnote 1, second reference, p. 184); originally published as "A Pathological Specimen Dating from the Lower Miocene Period," Contributions a l'étude des vertébrés miocènes de l'Égypte, Cairo, Survey Department, 1917. In this article Ruffer gave a valuable review of cases of arthritis deformans from the neolithic, bronze and Roman periods in western Europe, with references to the literature.

87. Rouillon, A.: Lésions osseuses préhistorique de la Vendée, Angers, 1923; reviewed in Anthropologie, Paris **36**: 153, 1926. Fürst, Carl (footnote 5). Teissier and Roque, in Gilbeert and Carnot: Nouveau traité de médicine, Paris, J. B. Baillière et fils, pt. 8, p. 121. Raymond, Paul (footnote 22). The two latter give other references to the literature.

88. Bull. Archeol. Survey Nubia, 1908, no. 2, has excellent illustrations.

in Nubia at the present day. The British investigators believed that many lesions of bone ascribed by earlier scientists to tuberculosis and other diseases were in reality produced by arthritis deformans (Wood-Jones,[13] Smith,[6] Ruffer [89]).

Arthritis deformans in ancient bones from American Indians was described nearly fifty years ago (Landon [15] and Whitney,[14] in excellent, judicious articles) and has often been observed since in Indian remains from North and South America, especially in skeletons from Peru (Hrdlička [16] and MacCurdy [17]). It has been reported in other ancient bones from Patagonia by Lehmann-Nitsche.[90]

The changes produced by arthritis deformans in earlier times do not seem to have been greatly different from those that one sees today, as far as one can determine from the numerous descriptions and illustrations that have been published. The spine (especially the lumbar spine) and the hip joint were the most common locations for it. Involvement of the knee joint was frequent. Other joints, such as the elbow, the shoulder, the sacro-iliac joint and that of the lower jaw, have also been found affected. The comparatively small number of reports of involvement of the fingers and the toes may be due to the ease with which these small bones may be lost or destroyed in old burial grounds; however, Wood-Jones observed several cases of it in the foot, hand and wrist. In old burial grounds, individual bones often cannot be identified as belonging to any particular skeleton. However, in a considerable number of cases, it could be shown that several or many joints in one skeleton were involved.

Bony union of adjacent articular surfaces (the proliferative type of Nichols and Richardson,[91] the atrophic type of some other authors) seems to have been comparatively rare (excepting, perhaps, the cases of Wood-Jones); while erosion, eburnation and deformity of the articular surfaces, with formation of bone in the capsule and ligaments (degenerative type of Nichols and Richardson, hypertrophic type of some other authors), was common. Immobility often resulted from the latter, especially in the spine. In the case of the spine, outgrowths from the edges of the bodies of the vertebrae, like irregular lips of bone, were common, as they are today; coalescence of such lips from adjacent vertebrae often produced immobility over larger or smaller portions of the spine. Many of the descriptions are not definite enough to let one determine the amount of ossification of the intervertebral

89. Ruffer, M. A., and Rietti, A.: J. Path. & Bact. 1912, vol. 16. Ruffer (footnote 1, second reference).

90. Lehmann-Nitsche: La arthritis deformans de los antiguos Patagones, Rev. del Museo de la Plata **11**:199, 1903. Verneau, R.: Les anciens Patagons, Paris, 1903, reviewed by Moodie, R. L.: Ann. M. History **10**:314, 1928.

91. Nichols and Richardson: Arthritis Deformans, J. M. Research **21** (n.s. **16**): 149, 1909.

disks; apparently such ossification was rare. Ruffer believed that irregularities occurring on the surfaces of bones at the point of attachment of muscles were due to ossification of the ends of tendons.

Ruffer studied sections of decalcified bones in some of his cases. He found the lesions superficial; the interior of the bone was normal; the bony thickenings were limited to the periosteum and neighboring tissues; the cartilage had atrophied, but signs of ossification of the cartilage were absent.

Smith (reported by Wood-Jones) observed in bones from Egypt a case of complete ankylosis of the spine, and there have been others like it. Almost all the skeletons of old persons from ancient Nubia showed spondylitis deformans. Smith and Dawson stated that rheumatoid arthritis "is par excellence the disease of the ancient Egyptians and Nubians." "The predynastic Nubian scarcely ever grew to adult life without experiencing some of its effects." Its manifestations were legion, of varied type and often of great severity. Ruffer [1] described many cases of it in bones from Egypt from various periods in its history, at great length and with meticulous care.

The most detailed account of arthritis deformans in the skeletal remains of American Indians is that of Hrdlička,[16] relating to the Peruvians. While the tibia and the humerus were sometimes involved, the spine and the hip joint were the commonest locations. He observed numerous cases of "mushroom head" femurs. There was sometimes considerable proliferation around the acetabulum; never synostosis.

Lehmann-Nitsche [90] found arthritis deformans present in about 4 per cent of ancient Patagonian skeletons. Rather curiously, the humerus and the elbow joint were somewhat more often affected than other parts. Apparently, the material came from between 100 and 150 skeletons. Contrary to his experience in the case of bones from Europe, he rarely found proliferations having the form of cauliflower in the bones from Patagonia. Polishing and eburnation were abundant. This he attributed to the fact that there was no rest for the damaged parts, owing to the nomadic life of these Indians.

It cannot be said that the study of arthritis deformans in the skeletal remains of ancient times throws any light on the causes of this calamitous disease, desirable as that would be. Race seems not to have had any influence: Negroes (Egypt), Indians, Caucasians of many nations, and even the Neandertal race all have been subject to it. All observers that mentioned the factor of age agreed that the bones indicated that adults, especially the elderly, were the persons affected. Several referred to the influence of exposure and moisture. Lehmann-Nitsche [90] alluded to the inclement climate of Patagonia, apparently in this connection. Gorjanović-Kramberger [84] ascribed the "cave gout" of his specimens of the Neandertal race to the conditions of life in their cave dwellings. Wood-Jones [13] laid stress on the fact that the people along

the Nile spend much time dabbling in the water, and that they have probably always done so; furthermore, periods of cold weather may alternate with intense heat.

Raymond [22] spoke of the frequency of arthritis in bones from caves, where moist conditions might be expected. (In France, rock shelters and caves have been used as dwelling places from the time of the Neandertal or Mousterian race down to and including the present day.) Ruffer [89] combated the theory that exposure to wet and cold operates as a cause of arthritis and implied that this theory is out of date; he reported on cases in skeletal remains of some of the dryest and hottest places in the world; he made the valuable suggestion that if an equal number of remains of man from other regions than Egypt and from the same early periods were to be examined, arthritis might be found to be equally common in them. Hrdlička [16] said of his Peruvian material that it showed plainly that the underlying cause was constitutional. It is difficult to see how diet could have been a cause when peoples of such diverse habits were affected. I do not know of any investigations that attempted to show how frequently arthritis was associated with diseases of the teeth in ancient times; such researches would be valuable, though difficult on account of the rarity with which complete or nearly complete skeletons can be secured. Bearing on this point, Keith [48] (who has had a large experience with skeletons of men of many races and from many periods) said that he had seen skeletons in which the teeth were affected and the joints sound, others in which the teeth were sound and the joints affected and others still in which both were affected. Rather curiously, it seems to me, few of the observers quoted said anything of injury as a factor in producing arthritis, or spoke of the difficulty that exists in distinguishing the effects of arthritis deformans from those of some old fractures, particularly at the head of the femur. Wood-Jones is one who did refer to this. I have met the difficulty in specimens from the dissecting room, when they were unaccompanied by histories of the cases.

Tumors.—Tumors produce such striking alterations in the forms of bones that a collector could not overlook them. One would, then, expect many reports on tumors of bone of ancient times, but the contrary is the case; there are few such reports. I have not been able to learn of any cases in bones older than those from Egypt, which I shall mention.

Osteoma: Small bony tumors are fairly common in the skulls of ancient Peruvians, most often in the upper surface. Osteomas or exostoses of the external auditory canal were mentioned by Whitney [14] as occurring in the skulls of Indians from all parts of the United States, from Canada, from Mexico and especially from Peru. He stated that the meatus might be entirely closed, and suggested a connection with the pressure on the posterior part of the skull that is

known to lead to flattening. Such exostoses are not an extreme rarity in patients of today. These bony growths about the ear in old skeletons have been described briefly by Hrdlička,[16] who has assembled a fine collection at the National Museum, Washington. He said, "These are generally hard osteomata, from one to three in number, just within. . . the orifice of the osseous meatus. . . . They were in no case seen to coalesce, and though they may almost close the meatus they were never seen to do this entirely. They were generally bilateral. Of 278 skulls, nineteen showed these exostoses, the specimens coming from the Chicama valley, Peru." Wood-Jones[13] referred to a case of the same kind from Nubia of the early Christian period. Moodie[92] reviewed other bone tumors collected by Hrdlička in Peru, and now housed in the museum at San Diego, Calif. He thought one or more of these tumors might be examples of meningioma or dural endothelioma invading the skull and provoking external hyperostosis.

Osteosarcoma: MacCurdy[17] described and gave a fine illustration of a large osteosarcoma of the cranium. Smith and Dawson[6] mentioned a large osteosarcoma of the femur and gave a good photograph of it. They also mentioned two of the end of the humerus. These were in skeletons from the cemetery near the Pyramids of Gizeh, dating from the fifth dynasty, and apparently were the most ancient examples of malignant tumors then recorded. Ruffer[93] described a large tumor, probably osteosarcoma, of the innominate bone, from Egypt, of about 200 to 300 A.D. It was impossible to tell whether this tumor was primary or secondary.

Carcinoma: Smith and Dawson[6] said of Egypt that "no evidences of true cancer occur until comparatively recent (Byzantine) times, when cases of malignant disease involving the base of the skull and sacrum suggest the presence of epithelioma of the naso-pharynx and rectum, respectively." Representations of tumors in human figures left by the ancient Greeks and Peruvians are referred to briefly on an ensuing page.

OBSERVATIONS ON ANCIENT TEETH

Ancient skulls have usually suffered a postmortem loss of teeth, and that is a serious obstacle to studies in this department. Anomalies, malformations and mutilations must be omitted from consideration in this article, which will limit itself to inflammatory and destructive diseases of the teeth and those of the jaws due to diseases of the teeth. Grinding down of the teeth (abrasion, attrition) produced by coarse and gritty food was frequent and often extreme in ancient races; exposure and infection of the pulp are said to have been common.

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92. Moodie, R. L.: Tumors of the Head among Pre-Columbian Peruvians, Ann. M. History **8**: 397, 1926.

93. Ruffer (footnote 1, second reference); J. Path. & Bact. **18**: 480, 1914.

The earliest known human jaws, the Piltdown jaw, which some comparative anatomists regard as that of an ape, and the Heidelberg jaw, exhibit some abrasion of the teeth but not any other disease.

Neandertal Teeth.—The roots of some specimens of teeth of Neandertal man are stout, and in the case of the molars and premolars the roots are fused (taurodont-Keith or prismatic-Kramberger). Fragments of several jaws belonging to this race at Krapina, in Croatia, were described by Gorjanović-Kramberger.[84] One of these has already been mentioned as showing evidence of arthritis and a fistula. Some of the teeth of this jaw were covered with tartar and the margin of the alveolar process presented a fine punctate appearance. From the description and the illustration, I should infer that there had been pyorrhea alveolaris (periodontoclasia). Pointlike depressions on some teeth may have resulted from caries (probably not); these appear to have been defects in the enamel. These bones are usually rated as rather early specimens of the Neandertal race, as is the lower jaw from Ehringsdorf, near Weimar, Germany, described by Virchow.[94] The latter specimen presents the results of a purulent process at the alveolar margin; the teeth are well worn and where the neighborhood of the pulp cavity has been reached "compensatory" (secondary) dentine has formed.

The skull of La Chapelle aux Saints in the Dordogne region, France, that of an elderly person, in which dental lesions were detected and described by Baudouin,[95] had lost many teeth during life; the jaws gave evidences of pyorrhea alveolaris ("gingivite expulsive" or "polyarthrite alvéolaire"), but not any trace of caries of the teeth.

Still another Neandertal skull, that of LaQuina, discovered by Henri Martin, belonging to a younger person, showed, according to Baudouin, deposits of tartar, especially on the molar teeth, and evidences of gingivitis between the molars, which he attributed to the use of toothpicks; dental caries was not present.

These instances are the only allusions to diseases of the teeth of the Neandertal race that I have seen. As far as these few specimens are concerned, pyorrhea alveolaris was common, tartar not rare, and dental caries unknown. However, in the case of several of the skulls of this race, I have not met with any reference to the presence or absence of disease of the teeth and jaw.

Martin [96] thought that the Neandertalers were carnivorous; Boule [96] that their diet was mixed. Roots, nuts, fruits and berries, it seems, would have been the most available forms of vegetable food for such a

94. Virchow, Hans: Die Unterkiefer von Ehringsdorf, etc., Ztschr. f. Ethnol. **47**: 447, 1915.

95. Baudouin, M.: Sem. méd. **32**: 170, 1912.

96. Martin, reviewed by Boule: Anthropologie **33**: 387, 1923.

savage people. Probably the diet varied with differing times and places. It is likely that the habits of those who dwelt in the center of Europe during the warm Riss-Würm interglacial period differed from the habits of the hunters of the cave bear in the high Alps. Charcoal found mingled with their implements indicates that this race used fire; whether or not they cooked their food must remain a matter of conjecture.

The Rhodesian skull from South Africa, generally regarded as related to the Neandertal type, of undeterminable but probably ancient date, has teeth that are well worn and that show marked caries and alveolar abscesses.[97]

Late-Paleolithic Teeth.—For the condition of the teeth in the late paleolithic or reindeer period, I have been able to find only a few notes. The jaws of the Old Man of Cro-Magnon (Aurignacian) indicate that he suffered from pyorrhea, as well as from bone cysts (MacCurdy[8]).

The specimen generally called the Chancelade skull (Magdalenien), now in the museum at Perigueux, southwestern France, had lost the teeth of the upper jaw during life, presumably from disease. One of the Obercassel skeletons (Magdalenien) had a fistula of the lower jaw[49] and the remains of purulent inflammation of the peridental membrane of a right molar, but not any caries. The teeth were well worn.

The only reference to dental caries occurring at this early period that I have seen is that of von Lenhossék,[98] who observed it in a brachycephalic skull from Nagysáp, Hungary, of the end of the diluvium. Von Lenhossék made the interesting suggestion that dental caries may be regarded as an epidemic disease, comparable to cholera and pest, which was brought into Europe from Asia by a brachycephalic race that invaded Europe just prior to the neolithic period.

At the meeting of the American Association for the Advancement of Science, in December, 1928, Fay Cooper Cole described two skeletons having root abscesses, loss of teeth during life and absorption of the alveoli. These skeletons were from shell heaps in Algeria, excavated by an expedition from Beloit College (U. S. A.), and the nature of the implements found with them indicates that they belonged to a period corresponding to the Aurignacian of France. To which level they may be properly referred cannot be fully established at present.

Neolithic and Later Teeth (Europe).—Baudouin[95] said that an authentic case of dental caries has not been shown in skeletal remains from any time prior to the neolithic period. With the introduction of agriculture, polished stone tools and what he calls civilization in general, dental caries appeared, although it was still ten times less

97. MacCurdy (footnote 8, vol. 1, p. 370); Keith (footnote 48, p. 398).

98. Von Lenhossék, M.: Die Zahnkaries Einst und Jetzt, Arch. f. Anthrop. **17:** 44, 1919.

frequent than at present. Substantially the same opinions were advanced in articles by Baudouin,[99] Rouillon and Baudouin [100] and Bouvet.[101]

Ruffer [102] reviewed the evidences of disease of the teeth in ancient Europe with numerous references to the literature. He alluded to skulls from a time apparently earlier than the neolithic period as having carious teeth. He concluded that, in the neolithic period itself in France, caries was neither common nor severe. Ruffer also commented on the occurrence of dental caries in England from the neolithic to the Anglo-Saxon period. Whether or not caries increased during the later periods is not clear (in general, it was found in about 20 per cent of the skulls). Keith [103] referred to a small group of neolithic skulls in Coldrum, England, as not having dental caries. Tratman,[104] describing a small series of teeth from a neolithic site in England, found that fifty-five of 100 teeth showed chronic periodontitis. There was a number of specimens that showed caries. Fürst [5] found carious teeth not rare in skeletal remains in Scandinavia (apparently of the neolithic period).

One of the first to make a survey of the teeth in a considerable number of ancient skulls was Mummery.[105] His work has been quoted by many subsequent writers. Mummery found, among skulls excavated in England, dental caries in two (2.94 per cent) of sixty-eight neolithic skulls (dolicocephalic); in seven (21.87 per cent) of thirty-two skulls of the bronze age; in twenty-four (40.67 per cent) of fifty-nine Yorkshire early dolicocephalic skulls; in nine (20.45 per cent) of forty-four ancient miscellaneous skulls; in forty-one (28.67 per cent) of 143 skulls of the Roman period, and in twelve (15.78 per cent) of seventy-six Anglo-Saxon skulls.

Von Lenhossék criticized Mummery's work, saying that it did not take account of teeth lost during life; such loss, he said, is chiefly due to caries. Apparently, von Lenhossék laid much less stress on pyorrhea alveolaris (periodontoclasia) and on dental abscess due to abrasion as causes of loss of teeth than did the British and American observers reviewed in this article. His own conclusions were based on the examination of more than 1,000 skulls in Budapest. It should be under-

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99. Baudouin, M.: Sem. dentaire **5**: 444, 1923; reviewed, Am. J. Phys. Anthrop. **8**: 342, 1925.

100. Rouillon and Baudouin: Presse Dentaire **26**:440, 1924; Rouillon (footnote 87).

101. Bouvet, reviewed, Anthropologie **34**:306, 1924.

102. Ruffer, M. A.: Study of Abnormalities and Pathology of Ancient Egyptian Teeth, Am. J. Phys. Anthrop. **3**:335, 1920; reprinted (footnote 1).

103. Keith (footnote 48, p. 13).

104. Tratman, reviewed by Moodie (footnote 52, p. 330).

105. Mummery, J. R.: The Relation of Dental Caries in the Ancient Inhabitants of Great Britain and Aboriginal Races, to Food and Social Conditions, Tr. Odontol. Soc. Great Britain **2**:7, 1870.

stood that jaws showing loss of teeth during life were counted by him as having caries. Von Lenhossék [98] found the frequency of caries increasing slightly from early to later periods in central Europe, as shown by skeletal remains; 85 per cent of the skulls from the Roman period, first century, and 83 per cent of those from the same period, fourth century, showing caries, as compared with 86 per cent of thirteenth century skulls, and 90 per cent of recent skulls. Schwerz [106] said that, according to the opinions of most writers, caries of the teeth is favored by culture and is comparatively rare under natural conditions. He examined the teeth of Alemannian Germans of about the tenth century. In 7,000 teeth, about 7 per cent were carious and tartar was frequent.

Ancient Egyptian and Nubian Teeth.—The most extensive reports on the teeth of ancient Egyptians and Nubians are those of Wood-Jones,[13] Smith,[13] Smith and Dawson [6] and Ruffer.[102] One who entered the same field early, as has been stated, was Mummery.[105] Thoma,[107] who studied the material in the Peabody Museum, Cambridge, Mass., found caries uncommon in skulls from ancient Egypt. Thoma encountered some bone abscesses.

Wearing or grinding down or attrition or abrasion of the teeth of Nubians from food that was coarse or contained much grit or sand was described by all the first three writers named. Such wearing away of the teeth was most marked in predynastic times and among the poorer people in later periods. Teeth were often worn down so as to expose the pulp cavities, and the frequent alveolar abscesses seen in such cases, as well as most of the dental disease seen in the archaic Egyptians and the poorer classes of the ancient Nubians, were attributed by Smith [13] to infection of the pulp. Wood-Jones described some severe alveolar abscesses with much destruction of bone.

Ruffer found evidence of the existence of pyorrhea in predynastic times and at later periods in Egypt and Nubia. He saw it in skulls of the twenty-fifth and twenty-sixth dynasties (from 750 to 500 B.C.) and in skulls of Copts of about 400 to 500 A.D. He saw it often among modern Egyptians. Ruffer regarded pyorrhea as the principal cause of the loss of teeth, and as a more common cause of alveolar abscesses than attrition; some such abscesses were caused by the decay of teeth.

Dental caries was rare in predynastic times, according to Wood-Jones;[13] it was more common during the New Empire; there were many examples in the Ptolemaic period; but it first became really common in the Byzantine period. Smith [13] also said that dental caries was exceedingly rare among the predynastic people, and that among the

106. Schwerz: Arch. f. Anthrop. **15**:41, 1917.

107. Thoma, K. H.: Oral Diseases in Ancient Nations and Tribes, J. Allied Dental Soc. New York **12**: 327, 1917.

poorer classes it never became common until modern times. But the ancient Egyptians of the wealthy classes who acquired habits of luxury suffered much from it. He mentioned that, in more than 500 skeletons of aristocrats of the time of the pyramid builders, formation of tartar, dental caries and alveolar abscesses were at least as common as they are in modern Europe. Ruffer seemed not to be certain that dental caries was less frequent in ancient times than it became later, but the facts cited by him indicate that such was the case. Ruffer apparently leaned toward the conclusion that there was some relation between diseases of the teeth and arthritis deformans, but he was cautious in expressing any definite opinion.

The observers mentioned agreed that evidence for the Egyptians ever filling cavities of teeth with gold or with anything else is lacking. In the rare examples of gold about the teeth, the gold was intended to be ornamental rather than useful. Nor did they find anything to indicate that extraction was practiced, or any other operative measure. Ruffer said also that there was nothing to point to the use of the toothbrush (which he characterized as an "instrument of torture").

Hooton [108] described a mandible from a tomb at Gizeh, of the Old Empire, in which an abscess had formed about the roots of the right first molar, the tooth being much worn and the pulp exposed. Two holes about 2.5 mm. in diameter led from the buccal surface of the jaw into the abscess cavity. They seemed to have been drilled and their nature was recognized by Thoma and Blumenthal. Todd [109] thought that the foramina in this specimen may have been natural and not produced by an operation.

Ancient Indian Teeth of North and South America.—Although American museums contain an immense amount of material that might give valuable information, the number of published articles on the teeth of the ancient Indians is not large. Hrdlička [110] described a morphologic peculiarity of the upper incisors of the Indians that is present in a large majority of persons of this race, and is seen to a smaller extent in other yellow-brown races: it depends on a hollow on the lingual surface making these incisors "shovel-shaped."

Hrdlička [111] found that dental caries occurred but rarely in skulls from Peru. He gave figures for 160 lower jaws from the Chicama Valley: of 708 teeth, 16 were carious. The absent teeth had been,

108. Hooton, E. A.: Harvard University African Studies. I. Oral Surgery in Egypt during the Old Empire, Cambridge, Harvard University Press, 1917, p. 29 (with good plates).

109. Todd, T. W.: Egyptian Medicine, A Critical Study of Recent Claims, Am. Anthrop. **23**: 460, 1921.

110. Hrdlička, A.: Am. J. Phys. Anthrop. **3**:467, 1920.

111. Hrdlička (footnote 16, p. 61).

for the most part, lost post mortem. MacCurdy,[112] in his studies on skeletal material from the highlands of Peru, found considerable dental disease, without evidence of any effort to treat such disease. Of 131 skulls, more than two thirds had decayed teeth. Of 1,259 teeth present, 194 (15.4 per cent) were decayed. The chief cause of the loss of teeth, aside from caries, seemed to him to have been pyorrhea alveolaris, which affected more than 13 per cent of all these jaws. Alveolar abscess was present in twenty-two cases, bone cyst in about ten, caries of the jaw in six and marked formation of tartar in thirteen. Moodie [113] recently began systematic studies on the diseases of ancient teeth from Peru. He has found pyorrhea prevalent, and has found that it resulted in an absorptive alveolar osteitis causing an appalling loss of teeth. He has thus far seen little evidence of a prevalence of caries. Thoma [107] described briefly alveolar abscesses and malformations of the teeth of Peruvians.

Hrdlička,[114] in a report on skeletal remains from Arkansas and Louisiana, said that decayed teeth, though not common, were fairly frequent: sixteen skulls did not show any teeth decayed or lost during life; thirty-nine had one or more decayed or lost during life. A good study was that of Leigh,[115] which should be read. Leigh chose skulls from four tribes of Indians, usually prehistoric, but at least living under natural conditions, free from the influence of white men. In sixty-eight skulls of Kentucky Algonquins, a sedentary people living on maize, and by hunting and fishing, he found marked attrition of the teeth; many alveolar abscesses; a little caries (twenty-eight lesions in 30 per cent of the specimens examined); some abscesses resulting from exposure of the pulp; periapical bone lesions (in 40 per cent) but not much periodontoclasia, which was chiefly senile, and comparatively rare calculi. In ninety-two skulls of the Sioux, buffalo hunters, eating fruit and vegetables to a limited extent, he found the least attrition of any, and little caries (slight lesions in ten of the ninety-two); alveolar abscesses (in 16 per cent) mostly from exposure of the pulp; and not much periodontoclasia (in 13 per cent). The teeth were clean. In 129 skulls of Arikara (a branch of the Sioux), mainly sedentary, living on maize pounded in stone mortars and by some hunting of the buffalo, he found much attrition; many alveolar abscesses; considerable caries (eighty-six lesions in 28 per cent of the skulls); alveolar abscesses (in 35 per cent), mostly from exposure of the pulp; and periodontoclasia (in 33 per cent). The teeth were dirty. One skull presented arthritis

112. MacCurdy (footnote 17, p. 276).

113. Moodie, R. L.: Studies in Paleodontology, J. Am. Dent. A. **15**:1826, 1928.

114. Hrdlička, A.: J. Acad. Nat. Sc. Philadelphia **14**:210, 1909.

115. Leigh, R. W.: Dental Pathology of Indian Tribes of Varied Environmental and Food Conditions, Am. J. Phys. Anthrop. **8**: 179, 1925.

of the temporomandibular joint. In 113 skulls of Zuni, a sedentary people, more advanced in cultural development than most tribes, living chiefly by planting, especially of maize, and a little hunting, he found moderate attrition of the teeth; caries (in 75 per cent); commonly accretions; much early loss of teeth; alveolar abscesses in 52 per cent, and periodontoclasia (in 56 per cent). There was more exposure of the pulp from caries than from abrasion. The teeth were dirty from calculi and stain.

As a further example of the fact that dental disease was not always rare among the Indians, I may mention material from a village site at Westfield, N. Y., pronounced by Arthur Parker to be early Iroquois (Erie).[116] These were a sedentary people, probably living largely on maize. The skulls were badly preserved and many teeth had dropped out. Sixty-six teeth present in the jaws were free from caries, ten showed caries, 103 loose teeth were free from caries and fifteen had caries. The cavities were large and the disease advanced. The teeth were nearly all those of adults. Many loose teeth showed marked thickening and even fusion of the roots in molars and bicuspids; these were not usually the carious ones. Sections of one such tooth showed the root thickening to be due to a new formation having the structure of cancellous bone; the root canal contained a large mass of secondary dentine. The root thickening was evidently caused by peridental inflammation.

The factors making for sound or unsound teeth, including the diet and the asserted influence of coarse food in cleaning the teeth, and the reported low incidence of dental caries among the Eskimos, who live on a diet of meat and fish, urgently call for further investigation, as does the possible influence of racial constitution. These studies are, however, outside the scope of this review.[117]

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116. I am indebted to R. P. Wright, of Erie, Pa., for an opportunity to see the specimens.

117. The following additional references give valuable information: Ritchie, S. G.: Rep. Canadian Arctic Expedition, 1913-1918; abstr., Am. J. Phys. Anthrop. **8**:343, 1925. Leigh, R. W.: Dental Pathology of the Eskimo, abstr., Am. J. Phys. Anthrop. **9**:400, 1926. Hellman, M.: Food and Teeth, Dental Cosmos, 1925, vol. 67, abstr., Am. J. Phys. Anthrop. **9**:398, 1926. Chappel, H. G.: Jaw and Teeth of the Ancient Hawaiians, abstr., Am. J. Phys. Anthrop. **11**:140, 1927. Black, D.: Human Skeletal Remains, etc., Geol. Survey China, abstr., Ann. M. History **8**:328, 1925. Gillett, H. W.: Contacts Between Archeology and Dental Research, Am. Anthrop. **29**:291, 1927. Dental Caries and Race, abstr., Am. J. Phys. Anthrop. **7**:405, 1924. Dr. W. D. Strong of the Field Museum, Chicago (in a personal communication to me), has compared the teeth in about forty Eskimo skulls, dating from about 1890 or earlier, with those in a series of 250 living Eskimos, all from northern Labrador. The amount of dental caries and loss of teeth was strikingly greater in the living Eskimos. There has probably been a gradual increase in the proportion of food of the kind used by the white race during the later period. These cases are now being studied and the results will be published later.

The practice of inlaying the teeth with jadeite, turquoise and the like for the purpose of ornament (not in treatment for disease) among the Mayas of Mexico and Central America and among the Indians of Ecuador was described by Van Rippen,[118] who gave a valuable review of ancient attempts at dental surgery.

OBSERVATIONS ON MUMMIES AND DRIED BODIES

The first published account treating of the study by modern methods of the anatomy and the histologic structure of the soft parts of ancient dried bodies was, so far as I can learn, that of Wilder.[119] Many more mummies have been destroyed or have been scraped so as to reveal the bony skeleton than have been saved for examination of the soft parts. Ruffer justly lamented this fact for Egyptian mummies. I suspect that it is equally true for those of Peru, of our southwestern states and of other regions, although the Egyptian material has been far more abundant. Smith and Dawson[6] gave vivid descriptions of the evidences of ancient tragedies in Egypt in the mummy of King Seknenrē, with its matted hair, wounds of the head and face and bones broken and cut by axe and spear, and in the body of a 16 year old girl, six months pregnant, whose wrists had been broken while she was defending herself from blows, one of which fractured her skull, the assailants presumably having been her own relatives. While the possibilities of such examinations are strictly limited, further study will surely bring to light valuable information. It is not unreasonable to expect that eggs of parasitic worms may be found in intestinal contents. Trichinosis might be detected in the mummified cats of Egypt, as well as in human bodies. While not much is to be hoped for in examinations for pathogenic bacteria, it seems to me not improbable that tubercle bacilli might be discovered in a body that had been rapidly dried, even after the lapse of thousands of years.

The Egyptian mummy, according to Smith and Dawson,[6] was usually prepared by removing the brain through the nose and by removing the abdominal and thoracic viscera, but not the heart, through an abdominal incision. The viscera were placed in "canopic jars" or, after being soaked in brine, were wrapped in cloths, with gums and sawdust, and little images of the gods, to be returned to the body later. The body was soaked in brine for seventy days, according to Herodotus, but for only half that time, according to Smith and Dawson, after which it was wrapped in linen bandages. The body cavity was filled with cloths, mud, sawdust and miscellaneous rubbish. Other details, such

118. Van Rippen: Dental Cosmos **59**:861, 1917.

119. Wilder, H. H.: The Restoration of Dried Tissues, with Especial Reference to Human Remains, Am. Anthrop. N. S. **6**:1, 1904.

as the use of artificial eyes and the external ornamentation, varied greatly at different periods, as did the addition of resins and other preservatives.

Peruvian mummies were sometimes eviscerated, but usually not. The body was bandaged, sometimes in a sitting posture with the knees flexed, sometimes extended. How much was done in the way of intentional embalming is not known. Balsam of Peru may have been applied to the bandages, which were of cotton. Apparently, the principal factor in the preservation of all mummies was a dry climate. Wood-Jones [13] found bodies from the predynastic period in Egypt (at least 5,500 years old), when artificial mummification was not practiced, which showed muscles and tendons, the skin and the hair, the nails and the eye and even the convolutions of the brain in good preservation. Mummies from Arizona and New Mexico in this country are merely dried bodies which the arid climate of the states has preserved. I do not know of any published accounts of examinations of Alaskan mummies; some specimens are now being studied at the American Museum of Natural History, New York, by Shapiro. Mummification in America and Australia was also described by Dawson.[119a]

Technic of Gross Examination of Mummies and Dried Bodies.—Wilder,[119] using material from Peru and from Utah in the United States, let the tissues swell for from twelve to forty-eight hours in a 1 to 3 per cent solution of potassium hydroxide, until they reached nearly their normal volume, then placed them in water, watching carefully that too much further swelling should not occur, and finally placed them in a 3 per cent solution of formaldehyde. In my laboratory, using similar material, my associates and I have found that merely soaking in a weak solution of formaldehyde (from 1 to 2 per cent) permits a satisfactory dissection. Ruffer,[120] working on Egyptian mummies, seems to have preferred, for dissection, carbonate of soda 2, solution of formaldehyde 0.5 and water 97. The part to be dissected was immersed in this solution for twenty-four hours or more. By any method, the fluid acquires a brownish-yellow color from the tissue.

Of course, everything depends on the completeness of the body and the state of preservation. It may be badly eaten by insects and worms. All observers agree that, in favorable cases, the muscles, the tendons, the large arteries, the veins and the nerves may be identified without much difficulty, and sometimes the cartilages of the larynx, trachea and bronchi, and the aorta. The condition of the great viscera of the thorax and of the abdomen varies greatly, but is usually much less satisfactory than that of the parts just enumerated; in the bodies of American Indians, the viscera may be dried to the consistency of thin mem-

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119a. Dawson, J.: Royal Anthrop. Inst. Great Britain & Ireland **58**:115, 1928.

120. Ruffer, M. A.: Note on the Histology of Egyptian Mummies, Brit. M. J., 1909, pt. 1, p. 1005; Remarks on the Histology and Pathology of Egyptian Mummies, Cairo Sc. J. **4**:1, 1910; Histological Studies on Egyptian Mummies, Mém. pres. a l'Inst. Égyptien, 1911, vol. 6; On Arterial Lesions Found in Egyptian Mummies, J. Path. & Bact. **15**:453, 1911; Pathological Notes on the Royal Mummies, Mitt. z. Gesch. d. Med. u. d. Naturw., 1914, vol. 13. These are the most important of Ruffer's numerous papers on mummies. All of them are reprinted in Ruffer (footnote 1, second reference).

branes. In Egyptian mummies, the viscera were largely removed during the preparation of the body, and the embalmers were apt to damage the aorta. The heart is found much shrunken and apt to crumble; however, its valves are sometimes recognizable. The brain in predynastic Egyptian bodies is sometimes found dried into a small, hard mass, giving, in miniature, the convolutions and the sulci. The eye is sometimes preserved, including the pupil. The hair, a resistant tissue, is usually well preserved; finger and toe nails often are. Wilder [119] noted that the ridges and folds of the skin of the hands and feet may be preserved. In the body of a baby Basket-Maker [121] that I have seen, prints from the foot and toes could easily have been made. Tattooing may show distinctly, as it did in the mummies of dancing girls (eleventh dynasty) tattooed with a pattern identical with that shown on faience figures of dancing girls belonging to about the same period.[121a] Exceptionally, such organs as the appendix, the cervix uteri, the thyroid gland, the papillae of the tongue, the ureter and the suprarenal gland have been recognizable. Smith and Dawson [6] told of the mummy of a woman, having the large breasts of lactation, buried along with her baby. Ulcers and wounds have been seen on Egyptian mummies, but it may be impossible to distinguish between antemortem wounds and the hacking of the body done in ancient times by grave robbers.

Technic of Histologic Examination.—By far the largest amount of work was done by Ruffer.[120] He placed the tissue in a preparation made of alcohol 30, water 50, and a 5 per cent solution of carbonate of soda 20; he sometimes used 1 per cent formaldehyde instead of alcohol and water. Fixation was completed in alcohol. He embedded the tissue in paraffin, and said that he obtained good sections, staining with hematoxylin and eosin, or with various aniline dyes.

In my laboratory, my associates and I have used fixation in formaldehyde, 1 to 2 per cent without any alkali, hoping to avoid damage to the finer structures. This may have been a mistake, for the tissues remained so hard that we could not make sections after embedding them in paraffin; usable sections were secured on embedding the tissue in celloidin. Wilder [119] and Wilson [122] have also worked on American mummy material. In general, the Egyptian mummies seem to have the structures of the large viscera better preserved than has been found to be the case in dried American bodies, as a rule, and the Egyptian mummies have given better results with nuclear stains. The failure of Egyptian mummies to show red corpuscles may be due to the soaking of the body in brine; the rapid drying of the American bodies probably made preservation of red corpuscles possible. The technic for preparing sections of bone has been discussed in connection with the diseases of bones.

Histology of Mummies and Dried Bodies.—All observers agree that the structure of common connective tissue, adipose tissue and cartilage is usually well preserved. Elastic tissue may or may not take specific stains. The cross striae of skeletal muscle fibers have been found both in Egyptian mummies and in American mummies; they may be seen well in teased preparations (fig. 11). Ruffer was successful in showing striations in heart muscle. The connective tissue framework of

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121. The Basket-Maker Indians preceded the builders of pueblos; their remains must have an antiquity of many centuries.

121a. Winlock: Bull. Metropolitan Museum of Art, New York, 1923, pt. 2, p. 26.

122. Wilson, G. E.: A Study in American Paleohistology, Am. Naturalist **61**: 555, 1927.

Fig. 11.—Skeletal muscle fibers showing cross striations from a Peruvian mummy, reprinted from H. U. Williams (Arch. Path. **4**:26 [July] 1927).

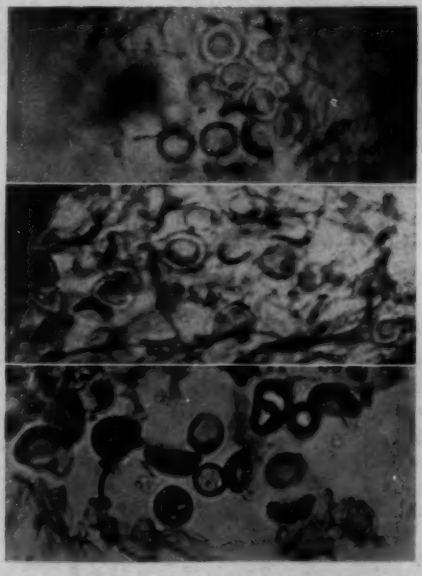

Fig. 12.—What are believed to be red corpuscles from a hemorrhage into the muscles of a Peruvian mummy, reprinted from H. U. Williams. (Arch. Path. **4**: 26 [July] 1927).

nerves may be all that remains, but Ruffer sometimes could demonstrate the medullary sheaths and even remnants of axis cylinders. As far as I can learn, it has not been possible to identify red corpuscles in Egyptian mummies. My associates and I found what we believe to be red corpuscles in the tissues of a Peruvian mummy (fig. 12). About six months later, Wilson reported finding them in Basket-Maker, Pueblo and Peruvian mummies. Rather curiously, the blood cells measured in my laboratory were sometimes larger than normal red corpuscles, being 8 or even 9 microns in diameter, while Wilson's cells averaged only 1.9 microns.

The blood vessels are usually well preserved, and the layers of their walls may be plainly made out, but the intima is not likely to be in good condition.

Ruffer was sometimes able to demonstrate the alveolar structure of the lungs, though the finer details were wanting. In the few American bodies that have been studied, the lungs were collapsed.

The liver cells have, as a rule, been found poorly preserved; the general arrangement that is characteristic of liver is about all that can be shown.

Little work has been done on the stomach and intestines, and that chiefly by Ruffer.[120] The layers of the walls may be recognizable, but the epithelium is wanting.

The only work on the kidneys that I know of was that of Ruffer, who was able to secure sections that showed the cortex, medulla, tubules and glomeruli, but not the finer details of these structures, even with high magnification.

Wilson [121] examined a thyroid gland from a Basket-Maker body. Only the connective tissue framework remained and epithelium was not present. Ruffer [120] had about the same result with testicles and mammary glands.

The epidermis is sometimes well preserved and sometimes wholly missing. Ruffer secured some excellent sections showing epidermis and even sweat glands. Simandl [122a] has published photographs of skin and cross-striated muscle from an Egyptian mummy. I have recently obtained good sections from the skin of the foot of a Basket-Maker baby from Arizona. These sections took some nuclear stain (fig. 13). Nuclei, in general, do not stain; although Ruffer had some success in demonstrating them, his attempts seem not to have yielded important results. Bacteria and mold fungi have often been observed in mummies and dried bodies. Ruffer was able to stain gram-positive and gram-negative organisms and spores. The certain occurrence of much putrefaction in these bodies makes it nearly impossible to connect such bacteria as may be seen with any disease process. But it seems to me that there is a good chance that tubercle bacilli and lepra bacilli may yet be found.

Serologic Tests.—The results reported from attempts to secure precipitin and anaphylactic reactions have been somewhat contradictory, with the weight of the evidence indicating negative results. The majority of these tests have been made on Egyptian mummies that had presumably undergone the prolonged soaking in brine. It would be worth while to make more tests on bodies that have only been dried, taking material that might be expected to contain plenty of dried blood or serum. A brief reference to such tests will be found in my article on Peruvian mummies.[123] If blood corpuscles could be obtained in sufficient quantities agglutination by serums of the various blood groups might be tried.

122a. Simandl: Anthropologie, Prague **6**:56, 1928.

123. Williams, H. U.: Gross and Microscopic Anatomy of Two Peruvian Mummies, Arch. Path. **4**:26 (July) 1927.

Moorleichen or Bog Bodies.—Something like mummification has been described in the case of the brown-stained, much flattened bodies known as "Moorleichen" that have been exhumed from sphagnum bogs in Schleswig-Holstein and other parts of northern Germany. Aichel [124] stated in his review that more than fifty such bodies had been recovered. As far as they can be dated, they belong to around 200 to 300 A.D.

Histology of Moorleichen or Bog Bodies.—The degree of preservation varied much in different cases. Wounds of the body were often visible. The acid swamp water had led to decalcification of the bones and solution of the muscles, while hair, nails, cartilage and connective tissue were often well preserved. The brain was sometimes recognizable as a brown mass containing cholesterol. The condition of the other viscera varied. In a female child described by Aichel, the fascia, vessels, tendons and nerves of the extremities were recognizable, also the liver, gallbladder, stomach and part of the intestine and the papillae of the tongue. The Moorleichen rarely revealed traces of cells. Collagen showed up well and

Fig. 13.—Low power magnification of a section of the skin from the sole of the foot of the dried body of a Basket-Maker baby about 10 months old. The Basket-Maker antedated the builders of the pueblos in New Mexico and Arizona, so that the body must have been many centuries old; no exact date can be fixed. Drs. Hooton, Kidder and Guernsey supplied me with the specimen.

outlined many structures; elastic tissue was not preserved; blood vessels contained masses that were probably blood corpuscles; the structure of medullated nerves was complete, except that axis cylinders were wanting; muscular tissue was missing; cartilage was in good condition; the bones were largely decalcified, but the minute structures were plainly visible, even to the bone corpuscles. Liver cells were not distinguishable; in the glands of the stomach, the epithelium was, in part, preserved. The tissues had suffered from the boring into them of the roots of plants, which had even made holes of fairly large size in the bones.

Pathologic Changes in Mummies, Dried Bodies and Moorleichen.—(The evidences of disease of the bones and the teeth have already been considered.) All the authorities that I have cited as having worked on

124. Aichel, Otto: Ueber Moorleichen, Anthrop. Anz. **4**:57, 1927 (with complete bibliography).

Egyptian mummies refer to calcifications or arteriosclerosis of the aorta and arteries. The principal types of arteriosclerosis seem to have been represented, as far as can be determined from the larger arteries. The disease was certainly not rare, and it occurred at various periods. Shattock [125] was apparently the first to report making sections of a sclerotic and calcified aorta. I have described thickening of the intima with calcification and an adherent thrombus in the posterior tibial artery of a Peruvian mummy [123] (fig. 14).

Wilder [119] mentioned carbonization of the lungs in Basket-Maker bodies. In a Basket-Maker baby (about 10 months old) being studied at the time of writing in my laboratory, the collapsed lung tissue already shows quantities of opaque granules that must, for the most part, rep-

Fig. 14.—Posterior tibial artery from a Peruvian mummy, showing a slight thickening of the intima with calcification, and a calcified thrombus; nitrate of silver and eosin stain; reprinted from H. U. Williams (Arch. Path. **4**:26 [July] 1927).

resent extraneous material that was inhaled during life. At the present time, the impalpable dust of the floors of the shelters where such bodies are exhumed is so easily stirred up as to impregnate the atmosphere, becoming a serious menace to scientific workers. The lung of the same baby had in it also several minute encapsulated areas of caseation. Gram-positive cocci, molds and organisms looking like ray-fungi were found in and near the areas. They may provisionally be considered as encapsulated abscesses, probably following broncho-

125. Shattock, S. G.: Lancet **1**: 319, 1909.

pneumonia. Wilson [122] also found silicosis in the lung of a Basket-Maker body, associated with a pleural adhesion. In Egyptian mummies Smith and Dawson [6] and Wood-Jones [13] observed pleural adhesions. Ruffer referred to anthracosis, and particularly to two cases of pneumonia. One of these was observed in a mummy of the Greek period, the lungs of which contained many bacilli that reminded him strongly of plague bacilli.

Apparently not many specimens of the stomach and intestines have been examined. The character of the food may be, in part, determined from their contents. Considering the alleged traditional prevalence of *Taenia saginata* in Abyssinia, one might expect to find tapeworm eggs in the intestines of ancient Nubians and Egyptians, if they ate beef. In general, the eggs of parasitic worms would stand a good chance of being preserved. Wood-Jones [13] mentioned prolapse of the intestine and vagina, also an old appendicitis.

Ruffer [120] described what seems to have been cirrhosis of the liver.

Smith and Dawson [6] referred to a single case of gallstones, the only one thus far found in Egyptian mummies.

Smith and Dawson [6] said that two cases of vesical calculus and three of stone in the kidney had been discovered in an examination of about 30,000 ancient Egyptian and Nubian bodies. One of these was found in a predynastic body. Wood-Jones [13] and Ruffer [120] also referred to vesical calculi; whether or not these two instances are included in the preceding statement is not clear. Ruffer observed a case of multiple abscesses of the kidney with many gram-negative bacilli. Williams [126] described a vesical calculus of mixed composition (fig. 15) from the body of a male Basket-Maker about 18 years old excavated in Arizona.

Vesicovaginal fistula is shown in the mummy of Princess Hehenit, eleventh dynasty. According to Derry,[127] "The injury is associated with an abnormally narrow pelvis, and it seems fair to suggest that it was probably produced during a difficult labor, and caused the death of the mother, who was quite young." Derry [128] also described a case of death of a Negress in childbirth discovered in remains from a Coptic cemetery. The fetal head was firmly wedged in the narrowed pelvis. The right sacro-iliac joint was missing (probably congenitally) and the right innominate bone was small, producing the distortion of the pelvis. The left sacro-iliac joint gaped widely, and there was overriding of the cranial bones of the fetus.

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126. Williams, G. D.: An Ancient Bladder Stone, J. A. M. A. **87**:941 (Sept. 18) 1926.

127. An account of this case, which will probably have been reported by Naquib Mahfouz before the present paper has been published, was given to me by Prof. Douglas E. Derry, of the Anatomy Department, Egyptian University, Cairo (personal communication).

128. Derry (footnote 77, p. 48).

The evidence of dystrophia adiposogenitalis or Froelich's syndrome in the case of King Akhenaton was related in the introduction.

Several of the royal mummies of Egypt showed appearances of the skin that may be all that is left of the eruption of some disease, but it was not possible to be certain; these evidences were mentioned by Smith [6] and Ruffer.[120] Baldness was frequent and comedones not rare. Several ulcers of the skin were found. Wilder [119] reported minute whitish granules on the skin of a Peruvian mummy, which appeared to be bacterial foci, and which might be the relics of some disease of the skin.

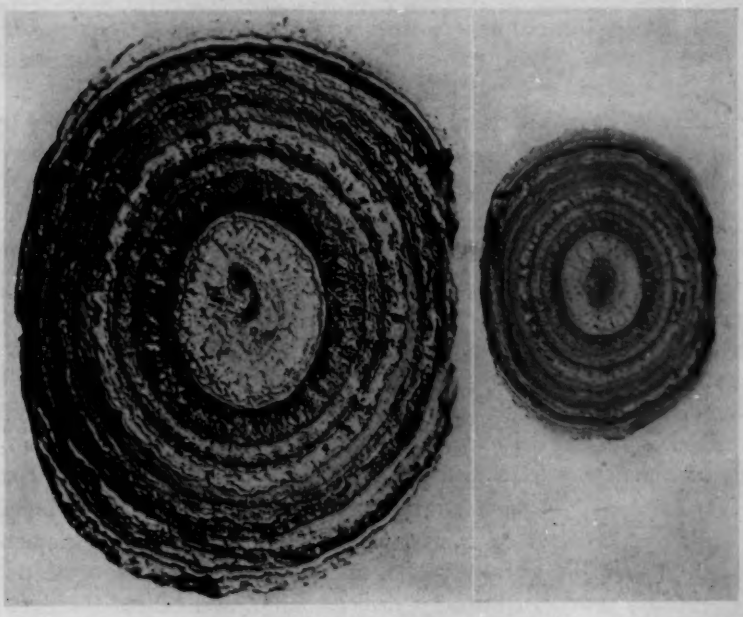

Fig. 15.—Calculus (natural size and magnified) from the urinary bladder of a Basket-Maker body. The photograph was sent me by Dr. George D. Williams, Washington University, St. Louis.

The occurrence of gout was observed by Wood-Jones [13] in an early Christian body from Egypt, which is described in considerable detail by Smith and Dawson.[6]

A single case of leprosy had been found up to 1924. This was in a body of early Christian date. It is mentioned by Smith and Derry [75] and by Smith and Dawson,[6] who give illustrations. Although leprosy bacilli were sought for, none were found. Innumerable cocci were present in the sections.

Rolleston [129] described the skeleton of a neolithic flint miner from Cissbury, England, with shortening of the bones of the left arm, which he attributed to poliomyelitis. The left humerous was $1\frac{3}{10}$ inches (3.25 cm.) and the left radius $\frac{8}{10}$ inch (2 cm.) shorter than the right. An Egyptian skeleton in the Archeological Museum of the University of Pennsylvania has shortening of the left femur to the extent of 8.2 cm., while the circumference of the shaft is somewhat reduced. This specimen was described by Mitchell,[130] who made a provisional diagnosis of poliomyelitis. The skeleton, excavated by Flinders Petrie, was said to belong to a period about 3700 B.C. The skeleton was that of an old man, and a slim staff buried with it was thought to have been used by him for balancing. It will be noted that the ancient man whose skeleton is next to be described also carried a staff.

The ancient Egyptian stela shown in figure 16 (described by Hamburger [131] and by Slomann [132]) may have been intended to represent the atrophy of one leg from poliomyelitis, though other causes of such atrophy must be entertained. Slomann contended also that the talipes equinus shown by the mummy of the Pharaoh Siptah, described and illustrated by Smith,[27] was due to poliomyelitis, probably occurring in childhood.

At the instance of Smith, a mummy of the twentieth dynasty (from 1250 to 1000 B.C.) showing an eruption resembling smallpox was studied by Ruffer and Ferguson,[133] who removed a portion of the skin for sectioning. Their modest assertion that the eruption resembles smallpox is justified by the illustrations of the gross appearance and of the sections as seen under the microscope. Numerous gram-positive bacilli and a few micrococci were demonstrated in the tissue. It was not supposed that these organisms played any part in the disease, but it was thought that they were present at or near the time of death. This case was criticised by Unna, to whom Ruffer [134] subsequently replied. In this second article, Ruffer seems to refer to the mummy in question as of the eighteenth dynasty. He mentioned a similar eruption on the mummy of Rameses Fifth, who belonged to the twentieth dynasty. Smith and Dawson [6] mentioned an eruption resembling smallpox on the body of Rameses Fifth. Ruffer's statements are not clear to me, but I should conclude that two such cases have been found in Egyptian mummies.

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129. Rolleston, G. H.: J. Anthrop. Inst. Great Britain & Ireland **7**:377, 1878.

130. Mitchell, J. K.: Tr. A. Am. Phys. **15**:134, 1900.

131. Hamburger, O.: Bull. Soc. franç. d'hist. de la méd. **10**:407, 1911.

132. Slomann, H. C.: Contributions a la paleopathologie Égyptienne, Bull. et mém. Soc. d'anthrop., Paris **8**: 62, 1927.

133. Ruffer, M. A., and Ferguson: J. Path. & Bact. **15**:1, 1911. Ruffer (footnote 1, second reference, p. 32).

134. Ruffer (footnote 120, fifth reference; footnote 1, second reference, p. 175).

A few enlarged spleens found by Ruffer may have been from ancient patients with malaria; the evidence for malaria is not powerful.

Tuberculosis has been discussed in connection with diseases of the bones.

Fig. 16.—A stela in polychrome from Egypt, of the period of the eighteenth dynasty (from 1580 to 1350 B.C.). The dimensions are 27 by 18 cm. It is thought to represent the results of acute poliomyelitis. The photograph was sent me by Dr. H. C. Slomann, of Copenhagen. The stela is in the Glyptothek of Ny Carlsberg in that city.

Wilder [119] spoke of a small species of mite (Acarina) that he observed in the stomach contents, uterus and nasal cavity of a Basket-Maker body associated with masses of what appeared to be blood corpuscles, suggesting a parasitic habit.

One of the most brilliant discoveries thus far made in paleohistology was achieved by Ruffer [135] when he found the eggs of *Schistosoma* (*Bilharzia*) in the kidneys of two mummies of the twentieth dynasty (from 1250 to 1000 B.C.). As the disease produced by *Schistosoma* is exceedingly common in Egypt at the present day, the demonstration of its occurrence at such a remote period in the same locality becomes one of extraordinary interest. Eggs of lice were also found by Ruffer, adhering to the hair of mummies.

PATHOLOGIC OBSERVATIONS IN ANCIENT ART

Art as depicting pathologic conditions of early times is considered at length in such works as that of Paul Richer. In the present article, it will be possible only to allude to a few of the more ancient examples.

Fig. 17.—Carving of a human figure excavated by Dr. G. Lalanne, of Bordeaux, at Laussel (Dordogne) in southwestern France, in 1911. It was carved in high relief on a block of limestone, about 67 cm. long. It belongs to the upper Aurignacian division of the late paleolithic period, and is probably around 25,000 years old. Figures of this kind are the oldest known portraits of man. A number of such have been found, frequently in the form of statuettes, over the region extending from southwestern France to Czechoslovakia. Such figures of steatopygous females are usually called venuses, and this one is the "Venus of Laussel."

In view of the fact that painters of the renaissance, as, for example, Velasquez, were fond of picturing persons with chondrodystrophy, cretinism and the like, similar efforts on the part of more primitive artists do not occasion surprise. The carvings, engravings and paintings of the cave artists of the reindeer period (from about 15,000 to 30,000

135. Ruffer (footnote 1, second reference, p. 18); Brit. M. J., Jan. 1, 1910, p. 16.

years ago), found especially in southern France and northern Spain, excite one's wonder and admiration by their accurate drawing and their lively spirit. MacCurdy,[8] who always succeeds in interpreting the work of primitive races with deep sympathy, remarked that men were artists before they became farmers. Unfortunately for our purposes, the cave artists chose for their models, for the most part, large mammals, like the reindeer, bear, mammoth and rhinoceros; they rarely drew their own kind. Several human figures in the form of steatopygous females have, however, been found (fig. 17). While not pathologic, the prominence of the secondary sexual characters is notable. Such figures, ironically called venuses, may have been symbolic, but they must also have been fashioned after models and may be considered as portraits; they are, besides, of unusual interest because, even if not flattering to our species, they are the earliest portraits of the human being known. The resemblance to the Hottentot female has often been remarked.[136]

The curious practice of mutilating the hands by amputation of one or more fingers has been employed by many primitive peoples as part of some ritual or sacrifice. Plain evidence that this custom prevailed among the cave artists is given on the walls of the caves where the hand was applied to the rock while some red coloring material was painted around it as with a stencil.[137]

Ancient Egyptian art reproduced figures of several pathologic conditions, which were described in an interesting article by Ruffer.[28] He gave illustrations of achondroplastic dwarfs 5,000 years old, of persons with talipes equinovarus 4,000 years old, and of other figures that he believed represent persons with Pott's disease and rickets, which are about 4,000 years old. The same subject was later reviewed by Dawson,[28] who also gave numerous illustrations.

Slomann [132] discussed several figures from Egyptian art, which he believed represent, respectively, Pott's disease of the spine, a humpback that may be rachitic, achondroplastic dwarfism, congenital dislocation of the hip and (fig. 16) what may well be the atrophy of one leg from poliomyelitis (eighteenth dynasty, from 1580 to 1350 B.C.). A good example of a pathologic condition shown in ancient Greek art is a statuette representing an ulcerating tumor of the breast.[138]

In America, the specimens of native art that attempt to delineate disease are largely confined to representations of the human body in pottery. The earliest note on such figures that I have met is in an

136. Zelisko: Einige Bermerkungen zur Frage der Steatopygie des Paleolithischen Menschen, abstr., Am. J. Phys. Anthrop. **7**:463, 1924.

137. This subject is thoroughly treated and examples from America shown by Smith, V. J.: Pub. Texas Folk-Lore Soc., 1925, no. 4.

138. Long, E. R.: History of Pathology, Baltimore, Williams & Wilkins Company, 1928.

article by Whitney [14] referring to images apparently meant to depict hunchbacks, from the "stone graves" of Tennessee.[139]

Spinden,[140] a believer in the American origin of yellow fever, gave drawings of certain pictures of the Aztecs, which he thought were meant to represent the "blood vomit" of yellow fever; they are not entirely convincing.

The pottery of Peru, as seen in the museums, shows many models of the human figure. Curious and unusual sexual practices that may be of interest to psychiatrists are sometimes vividly depicted. Much

Fig. 18.—Two clay figures (huacos) in the Peabody Museum, Cambridge, Mass., representing some form of ulcerative disease. They came from a prehistoric cemetery at Chimbote, Peru. The photographs were made for this article by Samuel J. Guernsey.

discussion has been occasioned by specimens of pottery (called huacos) that appear to show the results of ulcerative lesions of the face, especially about the nose, and others representing nodules of the face and, less often, of other parts of the body (fig. 18). Leprosy, lupus, syphilis, verruca peruana, yaws, gundu and uta all have been suggested as dis-

139. Moodie (footnote 18, p. 472, plate XCI) gave a photograph of such an image.

140. Spinden, H. J.: Yellow Fever, World's Work **43**: 169, 1921.

eases that the Peruvian artists intended to represent. Some specimens may have been intended to depict dwarfs, congenital malformations, intentional mutilations or the results of surgical operations, such as amputations. Recent opinion is disposed to regard many of the lesions shown on these images as attempts to represent uta, an ulcerative disease, usually of the face, especially of the region about the nose, which is still common in Peru. Strong [141] and his colleagues regarded uta as a form of leishmaniasis. A considerable literature has grown up in the discussions over these clay images from Peru.[142]

SUMMARY

The preceding review of what has been accomplished in studies on human paleopathology has led to certain conclusions, the most important of which I shall present in this brief summary.

Rickets seems to have been rare in ancient times. Rickets leaves changes in the bones so definite that, if present, they could hardly be overlooked. One encounters, however, a curious and striking condition, usually called symmetrical osteoporosis of the skull, which may be allied to rickets or scurvy or to some of the anemias of children, and which seems to occur rarely, if at all, among modern white peoples. Symmetrical osteoporosis was frequent among American Indians in the area where the cultivation of Indian corn or maize was most highly developed.

Arthritis deformans afflicted ancient peoples of both continents at least as often as it does today. The number of studies that consider a possible relation between arthritis deformans and diseases of or about the teeth in ancient times is so small as to be negligible. Such studies are much to be desired, but are made difficult by the fact that skulls are so often separated from the rest of the skeleton and many of the teeth in them lost post mortem. Several observers attributed arthritis deformans among the ancients to cold and moisture, but these observations were made, for the most part, about twenty years ago.

Arteriosclerosis prevailed in ancient Egypt in the same manner as it does among modern people, so far as the nature of the material permits us to form conclusions; a single case was found in a body from ancient Peru, among probably fewer than half a dozen bodies examined.

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141. Strong, R. P., et al.: Report of First Expedition to South America, Harvard School of Tropical Medicine, Cambridge, Harvard University Press, 1915, p. 175.

142. The earlier literature is largely given in the article of Lehmann-Nitsche, Robert: Patología en la Alfarería Peruana, Rev. Múseo de la Plata **11**: 29, 1902. More recent literature is given in the report of Strong (footnote 141) and Moodie (footnote 18, p. 488). One of the first in this field was Ashmead, A. S.: J. Cutan. & Ven. Dis. **13**:465, 1895; ibid. **14**:53, 1896; Am. Anthrop. N. S. **9**:738, 1907. Other papers by Ashmead are cited in Lehmann-Nitsche.

Anthracosis and silicosis are often found in ancient bodies. A few or single cases of the following diseases have also been demonstrated: pleural adhesions, pneumonia, cirrhosis of the liver, appendicitis, biliary and urinary calculi and gout.

Tumors of bone, in the form of small osteomas, were not rare. Osteosarcoma (or osteogenic sarcoma) must have been rare in ancient times. Even the great collections of bones from Egypt and from Peru and other parts of America have yielded only a small number of examples. This tumor often produces new growths of bone so startling that no collector could overlook them. Although osteosarcoma is perhaps not a common tumor today, every large museum of pathology has several modern specimens. It would be rash to say that this tumor has become more frequent in modern times, but it is proper to state the facts.

Carcinoma may erode the bones, especially of the face; the destruction so wrought could not fail to attract attention. I have been able to learn of only an insignificant number of ancient cases of this kind. However, erosion by cancer would often be difficult to distinguish from the postmortem erosion of bone by natural causes.

The teeth of ancient peoples were usually ground down or worn down by coarse food or dirt more than are the teeth of modern or civilized races. Dental abscesses and the condition known as pyorrhea alveolaris and as periodontoclasia and by a multitude of other names seem to have been at least as common in ancient times as they are at present; pyorrhea has been reported even as occurring in Neandertal man. Dental caries was stated by some investigators to have made its appearance first in the neolithic period. However, it should be remembered that the amount of material for study from the earliest periods is not to be compared with what is available from later periods; it is by no means certain that the last word has been said as to the date when men first began to have decay of teeth. In general, students seem to agree that dental caries was less common in the ancient periods than it has become in modern times, also that it became gradually more frequent as living conditions approached or merged into those of modern civilization. But what factor (or factors) in modern life is the one productive of dental caries has thus far defied analysis. The results to date certainly indicate that extensive studies on diet in relation to dental caries are needed.

Tuberculosis has been identified, in all reasonable probability, in Egyptian mummies that can be assigned to 2700 to 1100 B.C. The evidence in the scanty material from neolithic Europe is less convincing and that in the skeletal remains of ancient Americans must be called indecisive, leaning toward the negative.

Leprosy has been traced in pathologic material to but one probable case belonging to early Christian times in Egypt.

Poliomyelitis was probably present in neolithic Europe, and in Egypt as early as 3400 B.C.; the evidence is suggestive, but not conclusive.

Smallpox was the probable cause of certain lesions found in Egyptian mummies of 1250 to 1000 B.C.

Schistosomiasis was demonstrated in Egyptian bodies of 1250 to 1000 B.C.

Human paleopathology presents attractive possibilities for work in the future. Some of the large museums have great collections of ancient bones that have been examined only partially or not at all for conditions of disease. The teeth are particularly promising. Cooperation between archeologists, physical anthropologists and pathologists should bring about results useful to all three. The pathologists have already been useful to the archeologists and the historians in problems relating to the identification of bodies. While the possibilities in the examination of dried and mummified bodies are limited, they are fascinating. If the soft parts, as well as the bones, of all available mummies, could be studied by competent pathologists, as the bodies of patients dying in modern hospitals are studied, much information, I am confident, would be gained on disease in ancient times.

Notes and News

University News, Promotions, Resignations and Appointments.—Wiley Davis Forbus, associate in pathology in Johns Hopkins University, has been appointed professor of pathology in Duke University, Durham, N. C.

Novoa Santos of the University of Galicia has been made professor of general pathology in Central University, Madrid, Spain.

The William Wood Gerhard medal of the Philadelphia Pathological Society has been awarded to F. d'Herelle, now professor of bacteriology in Yale University, for his work on bacteriophage.

Werner Gunlach, Hamburg, Germany, has been called to the professorship of pathology in the University of Halle.

John R. Cuff has been appointed research fellow, and John A. Ferguson instructor in pathology, in the Harvard Medical School.

Maurice L. Cohan has been appointed instructor in pathology and bacteriology in the University of Illinois College of Medicine.

Kenneth Fowler has been appointed director of laboratories in the Jewish Hospital, St. Louis.

Charles H. Best has been appointed professor of physiology in the University of Toronto to succeed J. J. R. McLeod who becomes professor of physiology in the University of Aberdeen.

Eugene L. Opie, director of the department of pathology in the University of Pennsylvania and of the laboratory of the Henry Phipps Institute, has been elected a member of the board of scientific directors of the Rockefeller Institute for Medical Research.

Katsusaburo Yamagiwa of the University of Tokyo has been awarded the Sophie A. Nordhoff-Jung Cancer Prize of $1,000 for his work on producing cancer in animals by means of tar. This is the third award of this prize, the previous recipients being the late Johannes Fibiger and Otto Warburg.

Ernest M. Hall has been appointed professor of pathology and bacteriology in the school of medicine of the University of Southern California, Los Angeles.

Leonor Michaelis of the Johns Hopkins University School of Medicine, has been appointed to the staff, with the title of member, of the Rockefeller Institute for Medical Research, New York.

Frederick W. Andrewes Honored.—Number 2, volume 32, of the *Journal of Pathology and Bacteriology* (April, 1929) is dedicated to Frederick William Andrewes on his seventieth birthday, March 31, 1929.

American Association of Pathologists and Bacteriologists.—The next meeting of this association will be held in New York, April 17 and 18, 1930.

Formation of Pathologic Society in San Francisco.—On April 22, 1929, an organization to be known as the San Francisco Pathologic Society was formed. A constitution and by-laws were adopted and the following officers were elected: president, William Ophüls; vice president, G. Y. Rusk; secretary-treasurer, Z. E. Bolin; executive committee, C. L. Connor, A. M. Moody and W. T. Cummins. It is interesting to note that a similar society was formed in 1851 in San Francisco and that this was one of the earliest medical organizations on the Pacific coast.

Death of Alexander Ogston.—Alexander Ogston, regius professor of surgery in the University of Aberdeen and a pioneer student of the bacteriology of inflammation and suppuration, to whom is owed the discovery of staphylococci, died on Feb. 1, 1929, at the age of 84 years. He introduced the name *Staphylococcus*. His name will have "a permanent place in the history of pathogenic bacteriology in its classical period."

Pathologic Society of Great Britain and Ireland.—The next meeting of this society will be held at Cambridge on July 5 and 6, 1929.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

EXPERIMENTAL PURPURA WITH ANTI-PLATELET SERA. ROSCOE R. HYDE, Am. J. Hyg. **8**:870, 1928.

Miliary purpura was produced on the skin of a guinea-pig by the intravenous administration of antiplatelet serum, the attack being initiated by shaving the skin over the abdomen. A deficiency in complement did not prevent the appearance of the purpura. Subjection of the antiplatelet serum to repeated absorptions with excessive amounts of boiled corpuscles without effect would indicate that the antibody is evidently not of the nature of a heterophile immune body. Purpura produced by antiplatelet serum is intensified in animals whose blood has been previously rendered incoagulable by the anti-coagulant heparin.

PEARL ZEEK.

THE EQUATION EXPRESSING THE EXCRETION OF A DIURETIC AND ITS RELATION TO DIFFUSION PROCESSES. E. J. CONWAY, Am. J. Physiol. **88**:1, 1929.

Proceeding from the theory that the total volume of urine comes from the glomeruli, being similar in composition to a blood filtrate, and that all substances appearing in the urine in greater concentration than in the blood are added by diffusion from the tubule cells, the author develops theoretical formulas to cover various relations between the plasma and urine. Of the several formulas presented, perhaps the one of most direct application is:

$$\sqrt{\frac{1}{t}\,\frac{Cu}{C_B}} = \text{constant}$$

which shows the diffusion-secretion relations of urea excretion in the human being; this has been shown by statistical analysis to be the true relation for volumes of urine up to approximately 4 liters per day. Evidence is presented to show that sulphates and phosphates follow the same relationship. As to the constancy of the relationship of the output of urea to the blood concentration at high volumes of urine and normal blood concentrations, the author presents reasons to indicate that this may be due to the fact that available renal energy reaches a limit with high volumes of urine, approximately 4 liters a day.

H. E. EGGERS.

THE EQUATION EXPRESSING THE EXCRETION OF A DIURETIC AND ITS RELATION TO DIFFUSION PROCESSES. E. J. CONWAY and F. KANE, Am. J. Physiol. **88**:29, 1929.

The threshold value of chloride was found by the authors to be 0.574 per cent for the rabbit, a figure practically identical with that obtained for the human being by McLean. ("Threshold value" is regarded as the highest limit of plasma concentration at which the urinary concentration is less than plasma concentration for all rates of flow of urine.) This value was not affected by injections of dextrose even in large amounts, but was increased by the injection of sulphate or of urea. The theory of the chloride excretion, advocated by the authors, is that its concentration in the urine is determined, after primary discharge, by the rate and direction of chloride diffusion during the passage of the urine along the tubular tracts, the water output being regulated solely at the glomerulus. From premises based on this theory, they show that the rate of absorption of sodium chloride is directly proportional to $(T - C_B)$, when T is threshold value and C_B is blood concentration, and that with a constant value of blood concentration, the rate of absorption is proportional to t, t being the time of a unit volume of urine. These relationships are closely analogous to the diffusion of iodine from chloroform into aqueous solutions of potassium iodide, and are in harmony with facts established in other lines of renal investigation.

H. E. EGGERS.

EFFECT OF CORPUS LUTEUM AND OVARIAN EXTRACTS ON THE OESTRUS OF THE GUINEA-PIG. D. I. MACHT, A. E. STICKELS and D. L. STECKINGER, Am. J. Physiol. **88**:65, 1929.

A study was made of the effects of specially prepared water extracts of corpus luteum on the estrus cycle of the guinea-pig by means of the vaginal smear method. The extracts were found to produce inhibition of estrus, and this inhibition was accompanied by characteristic histologic observations in the ovaries, consisting of a diminution of maturing follicles and a tendency toward the development of atretic follicles. The injection of other glandular extracts was without this effect; only placental extracts tended toward a similar action. It was observed that in the presence of even slight pyogenic infection, the estrus was affected to the point where the sexual cycle became decidedly irregular.

H. E. EGGERS.

THE EFFECT OF DAILY TRANSPLANTS OF THE ANTERIOR LOBE FROM GONADECTOMIZED RATS ON IMMATURE TEST ANIMALS. E. T. ENGLE, Am. J. Physiol. **88**:101, 1929.

On transplanting daily the fresh anterior lobe of the pituitary from gonadectomized rats into immature females, the ovarian response was definitely greater than when the similar tissue from normal rats was transplanted. There was no sex difference in respect to the gonadectomized animals, and no significant differences as regards the age of the donor at the time of gonadectomy. The ovarian response was so much greater that in addition to the increased size of the pituitary in the gonadectomized animal, it is believed that there was an additional storage of the gonadal stimulating factor. These results are explained by the author as indicating the existence of a gonadal-pituitary "releasing mechanism," the presence of the gonad being of prime importance in releasing the hormone from its site of origin.

H. E. EGGERS.

THE EFFECT OF MASSAGING THE THYROID ON THE BASAL METABOLIC RATE OF DOGS. O. B. DE COUTO-E-SILVA and C. S. SMITH, Am. J. Physiol. **88**:183, 1929.

In trained dogs, without anesthesia and under conditions as nearly physiologic as possible, external massage of the thyroid gland for half an hour was found to produce no immediate and little, if any, delayed increase in the metabolic rate.

H. E. EGGERS.

EXPERIMENTAL STUDIES ON DENERVATED LUNGS. R. FONTAINE and L. G. HERRMANN, Arch. Surg. **16**:1153, 1928.

In the dog, partial denervation of one lung is a safe procedure, and can be accomplished by unilateral resection of the first four thoracic sympathetic ganglions and removal of the ansa vieussenii and the stellate ganglion, together with the resection of the portion of the vago-sympathetic trunk which has included in it the middle cervical ganglion. The authors find that section of these nerves that lead to one lung does not cause any change in the frequency or character of the respiratory movements. Such a lung shows no difference in reaction to stimuli from the intact one. The carbon dioxide combining power of the plasma is not affected. The occurrence of massive collapse is not prevented.

N. ENZER.

PROBABLE EFFECT OF PANCREATIC JUICE ON THE REGULATION OF GASTRIC ACID. R. ELMAN, Arch. Surg. **16**:1256, 1928.

During drainage of the total pancreatic juice the gastric contents contain far higher total and combined acid than normal. Acid solutions introduced into the stomach of animals during such drainage provoke an intense increase in the flow

of pancreatic juice to the outside. Drainage of pancreatic juice is accompanied by regurgitation of the intestinal contents into the stomach. The injected acid is not neutralized as in the normal animal. The author states that this evidence supports the hypothesis that the reduction of gastric acid results from the reflux of alkaline pancreatic juice into the stomach.

N. Enzer.

Influence of Light Environment on Normal Rabbits. Wade H. Brown, J. Exper. Med. **49**:103, 1929.

A series of experiments was carried out for the purpose of determining whether a light environment comprising radiations of comparatively long wave length and of only a small amount of energy was capable of affecting the chemical equilibrium of the blood as indicated by the calcium and inorganic phosphorus content of the blood of normal rabbits. A study was made of effects produced by prolonged exposure to fixed environmental conditions (neon light and darkness) as compared with a varying environment of diffuse, filtered sunlight, and by a change from one environment to another. It was found that the chemical equilibrium of the blood was definitely affected by the conditions employed and that the effects produced could be correlated with differences in organic constitution on the one hand, and on the other, with certain differences in the functional activity of the same animals, involving nutrition, growth and the proliferative activity of hair follicles.

Author's Summary.

Calcium and Phosphorus Metabolism. Walter Bauer, Joseph C. Aub and Fuller Albright, J. Exper. Med. **49**:145, 1929.

The trabeculae of the bone are easily depleted by the prolonged administration of parathyroid extract-Collip, long continued negative calcium balance and growth. A long continued high calcium diet results in a rapid accumulation of the trabeculae. Alizarin red, as has previously been shown in the literature, is deposited in newly formed bone. Its use has made clear that easily mobilizable calcium is not deposited in the shafts of adult animals, but in the trabeculae of bone. The bone trabeculae therefore serve as the storehouse of readily available calcium. The shafts have a slow, progressive exchange of inorganic salts and are not influenced except in the case of unusual demands of the body. It is suggested that the absence of trabeculae in premature infants and their depletion at the end of four months in a normal baby might well be an etiologic factor in rickets. In these observations, the administration of parathyroid extract-Collip to growing or adult cats has been without effect. Daily injections of parathyroid extract-Collip in growing rats result in an increased number of trabeculae and smaller bones.

Authors' Summary.

The Chemical Nature of the Substances Required for Cell Multiplication. Lillian E. Baker, J. Exper. Med. **49**:163, 1929.

It has been shown that the ash of liver, hemoglobin and glutathione each exerts a slight beneficial effect on the growth of sarcomatous fibroblasts of the rat, or on the condition of their cells when cultivated in a synthetic medium. The addition of all three of these substances, or of only glutathione and hemoglobin, to a mixture of casein digest, glycocoll and nucleic acid gives a medium in which sarcomatous fibroblasts of the rat proliferate for a considerable time as rapidly as in embryo juice. The mixture is not as adequate a nutritive medium as embryo juice, for after a time dead cells are found surrounding the central fragment of the culture, and after several passages the growth becomes thinner. The hypothesis is suggested that glutathione and hemoglobin may function not only by regulating the respiration and oxidation reduction reactions within the cell, but also by regulating the oxidation-reduction potential of the medium. It is suggested that the failure to obtain growth of fibroblasts in mixtures of amino-acids or of

the products of complete acid hydrolysis of proteins is in part due to the absence of glutathione, and that this substance is not synthetized by fibroblasts. The growth of normal fibroblasts of the rat is also increased by the addition of the aforementioned substances to a synthetic medium.

AUTHOR'S SUMMARY.

BLOOD REGENERATION IN SEVERE ANEMIA. F. S. ROBSCHEIT-ROBBINS and G. H. WHIPPLE, J. Exper. Med. **49**:215, 1929.

A liver extract, no. 343, N. N. R., known to be fully potent in pernicious anemia shows only from 10 to 20 per cent of the potency of whole liver when fed to dogs for severe continuous secondary anemia due to bleeding. There are wide individual variations which are not noted when whole liver is fed. Supplementing this liver extract with whole bile does not modify the reaction. Supplementing the liver extract with liver ash or apricot ash shows the sum of the two expected feeding reactions. When this liver extract is supplemented with small amounts of whole liver (from 50 to 100 Gm.) there is a production of hemoglobin and red cells in excess of the sum of the expected separate reactions. Whether the liver extract increases the potency of the whole liver or vice versa, it suggests similar possibilities in various human secondary anemias. Liver and kidney fractions of varied types deserve much study to ascertain their effect on widely divergent types of anemias in human beings.

AUTHORS' SUMMARY.

THE RELATION OF FREQUENCY TO THE PHYSIOLOGICAL EFFECTS OF ULTRA-HIGH FREQUENCY CURRENTS. RONALD V. CHRISTIE and ALFRED L. LOOMIS, J. Exper. Med. **49**:303, 1929.

The biologic effects of electromagnetic waves emitted by a vacuum tube oscillator have been studied at frequencies ranging from 8,300,000 to 158,000,000 cycles per second (a wave length of from 1.9 to 38 meters). The effects produced on animals can be fully explained on the basis of the heat generated by high frequency currents which are induced in them. No evidence was obtained to support the theory that certain wave lengths have a specific action on living cells. At frequencies below 50,000,000 cycles, the effect of these radiations on animals is proportionate to the intensity of the electromagnetic field. As the frequency is increased beyond this point, the amount of induced current is diminished and the apparent lethality of the radiation is decreased. This can be explained by changes occurring in the dielectric properties of tissues at low wave lengths.

AUTHORS' SUMMARY.

INTERMEDIATE METABOLISM OF FAT IN HEPATIC DISEASES. C. CASSANO, Arch. di pat. e clin. med. **7**:441, 1928.

In all the cases of hepatic diseases studied, it was established that the fat metabolism is greatly disturbed, and that the resulting lipemic conditions are usually due to a retardation of the intermediate fat metabolism. The increase of the neutral fats and the decrease of the phosphatids is a constant result. There are no special types of hepatic disturbances of fat metabolism that would correspond to a definite diseased condition of the liver; the disturbances reveal only the degree but not the character of the hepatic lesion. The functional insufficiency of the liver in metabolism of fats commonly varies in intensity and often exhibits new pictures of metabolic disturbance. The fatty acids appear increased, indicating the inability of the liver to split them. They are partly bound with cholesterols, forming cholesterol esters. As a rule, there is an increase in cholesterols which appears necessary in order to assure the synthesis and the diuresis of the phosphatides. It seems that the cholesterols, which are regulated by the liver, neutralize the toxic action of the fatty acids. The anatomic and functional lesions of the liver are accompanied by varied disturbances in the chemical behavior of the cholesterols, phosphatides and fatty acids.

E. L. MILOSLAVICH.

FACTORS ASSISTING THE HEART IN ITS WORK AS A SUCTION AND PRESSURE PUMP. G. HAUFFE, Virchows Arch. f. path. Anat. **266**:18, 1927.

The circulation of the blood is maintained by the heart alone, through the opposing movements of the walls in the auricles and ventricles. This opposing movement is achieved by the enclosure of the heart in the pericardium. The system of the pericardium and heart acts as a suction and pressure pump, while the conduction system maintains proper correlation between the two forces. In the circulating blood, the movement is influenced by various secondary factors, activated by the central motor, the elasticity of the vessel walls, valves in the veins, etc. These maintain the original speed as nearly as possible to the end, and disappear when the work is accomplished. Systolic stretching of the aorta does not occur, and the capillaries do not act as a resistance to the blood stream. Distribution of the blood in the service of respiration of the cells is effected by certain organic forces. These forces perform the work of changing the width of the blood vessels, and therewith are dissipated. They, as well as the other factors assisting the circulation, must be set in motion afresh each time by the heart through its suction activity.

B. R. LOVETT.

DIFFERENCES IN RESISTANCE OF THE FEMALE AND MALE SEX GLANDS. H. V. KLEIN, Virchows Arch. f. path. Anat. **266**:18, 1927.

The longer persistence of sexual activity in the testis than in the ovary may be explained by differences in the resistance of the two glands, or on the basis of inherited, sex-limited characteristics. From the literature, it appears that the ovary is in general more resistant than the testis to operative procedures, partial removal, thermal and chemical injuries. The regenerative power of the ovary is greater, and in autotransplants it lasts longer.

The author's experiments with autotransplants in rabbits and guinea-pigs showed, in the case of males, marked individual differences in the results. In some, the sexual function of the transplants persisted, while in others it was completely lost. In one female there was persistence of function, although the transplanted organ disappeared completely. Roentgen irradiation of the ovaries was followed by sexual hyperactivity, probably due to premature rupture of follicles with the escape of sex hormone into the blood. No analogous result occurred in males. Klein concluded that the ovary is more resistant than the testis. Its cessation of function after the fifth decade cannot be explained entirely on the teleologic basis, that woman is not fitted to go through pregnancy after this time, but belongs to the picture of the genotype of the human female.

B. R. LOVETT.

Pathologic Anatomy

INTUSSUSCEPTION DUE TO PAPILLOMA IN A CHILD OF TWENTY-ONE MONTHS. EDWARD J. LAMB, Am. J. Dis. Child. **36**:1017, 1928.

Papillomas of the small intestine are rare and intussusception caused by papilloma is of rare occurrence. When polypoid growths occur in the intestine, they are apt to be multiple. Recovery of an infant after resection for invagination ileus is a surgical curiosity.

AUTHOR'S SUMMARY.

A CASE OF PYOSALPINX CAUSED BY OXYURIS VERMICULARIS COMPLICATED BY TORSION OF THE OVIDUCT. W. SMITH and J. DENTON, Am. J. Obst. & Gynec. **16**:205, 1928.

This interesting case occurred in a nullipara, 23 years of age, who for six weeks had pain in the lower right part of the abdomen, which grew progressively worse. The blood count and eosinophil ratio were practically normal. At laparotomy the right tube was found twisted from left to right, distended and cystic; the left tube

was likewise enlarged. The wall of the left tube was enlarged due particularly to a thickening of the mucosa and submucosa, and here an extensive infiltration of round cells could be seen. There were numerous foreign body giant cells close to sections of the worm. The similarity of this process to tuberculous reaction of the fallopian tube was observed.

A. J. KOBAK.

ECTOPIC CORPORA LUTEA. VERA B. DOLGOPOL, Am. J. Obst. & Gynec. **16**:218, 1928.

The term "ectopic corpora lutea" is proposed by Dolgopol for corpora lutea that becomes partially or totally separated from the ovary. The author added six cases to the twenty-four previously described in the literature and pointed out an omission of this condition in the English literature. Since the cases described by the author all occurred in five months, with a similar frequency cited in another report, it was suggested that this condition may be overlooked by surgeons. No untoward symptoms were associated with this condition.

A. J. KOBAK.

ENDOMETRIOSIS FOLLOWING SALPINGECTOMY. JOHN A. SAMPSON, Am. J. Obst. & Gynec. **16**:461, 1928.

Sampson demonstrated that salpingectomies, as usually practiced, injure the endosalpinx, and the latter may subsequently invade the tubal stumps and the structures adherent to it. This was shown in thirty of thirty-six patients whose fallopian tubes had been operated on. Endometriosis grew out of the misplaced and traumatized tubal mucosa as sprouts or seedlings near the tubal stumps and invaded the uterine cornu. Endometriosis of the tissues of the broad ligament was continuous with the endometrial-like lesions on the median side of the ovary (three cases), or with the scar of the abdominal wall in two cases of ventrofixation of the uterus. The author believes that the ordinary surgical technic carelessly transplants tubal epithelium to other anatomic sites or within the incision. The misplaced tubal mucosa at times retains its original structure or assumes the structure and function of the uterine mucosa. The term endometriosis used for invasion by either tubal or uterine mucosa was admitted to be inadequate but was preferred to other names proposed by investigators of ectopic müllarian tissue.

A. J. KOBAK.

CYCLICAL AND OTHER VARIATIONS IN THE TUBAL EPITHELIUM. E. NOVAK and H. S. EVERETT, Am. J. Obst. & Gynec. **16**:499, 1928.

Novak and Everett show that the tubal epithelium exhibits definite cyclical changes comparable with those of the endometrium, but does not participate in the bleeding of the menstrual process. Two types of cells are chiefly concerned with this cycle. For example, the ciliated cells which reach the height of development during the interval period, and become smaller during the premenstruum, and the nonciliated or "secretory" cell which exhibits greatest development and activity in the premenstruum. During the menstrual phase the ciliated cells become smaller and the nonciliated cells empty their cytoplasmic contents. During the postmenstruum period, both types of cells are smallest but growth rapidly recurs up to the interval phase. A third type of cell designated as "Peg" cells are described, but their function and origin are rather obscure. In prepuberty, the epithelium is low, but both the ciliated and the nonciliated cells are present. The epithelium during the menopause remains high for a number of years. The corpus luteum was considered responsible for cyclic variation in the tubal epithelium similar to that evoked in the endometrium. The tubal epithelium in patients having an "essential" hyperplasia of the endometrium was studied in a limited number of patients. The epithelium in general was tall and rather narrow with a diminution of secretory activity of the nonciliated cells. This is to be expected since this hyperplasia is functional and based on an excess of follicle stimulus with diminished corpus luteum influence, whereas the cyclic changes were attributed to the physiologic response of the two chief types of cells to both the follicle and the corpus luteum.

A. J. KOBAK.

STUDIES IN ACROMEGALY (FUGITIVE ACROMEGALY). PERCIVAL BAILEY and HARVEY CUSHING, Am. J. Path. **4**:545, 1928.

That whereas a highly chromophilic type of adenoma, the cells of which are heavily laden with alpha granules, characterizes outspoken acromegalic hyperpituitarism, and whereas an adenoma of purely chromophobe type with nongranular cells is commonly associated with adult hypopituitarism, there is an intermediary group of cases in which traces of these opposed symptoms have apparently been present from the outset and which are associated with a histologically distinctive adenoma with cells of fetal type having sparse, peripherally disposed granules. Since this intermediary syndrome is distinguishable clinically from the more common hypopituitary state by recognizable traces of hyperpituitarism, we find it convenient to refer to the disorder as fugitive acromegaly.

AUTHORS' SUMMARY.

EXPERIMENTAL GLOMERULONEPHRITIS PRODUCED BY INTRARENAL TUBERCULIN REACTIONS. ESMOND R. LONG and LUCY L. FINNER, Am. J. Path. **4**:571, 1928.

The experiments described demonstrate that, by the injection of tuberculin into the kidneys of swine made sensitive to this substance by the presence of mild tuberculosis, it is possible to produce a diffuse inflammation of the kidneys that may properly be considered an acute glomerulonephritis. Moreover, this effect is the result of a true tuberculin reaction, as it does not occur following the perfusion of the kidney of a nontuberculous animal with tuberculin. We may therefore add the intrarenal tuberculin reaction to the intradermic, conjunctival, intratesticular and other anatomic varieties of tuberculin reaction previously described. The immediate reaction in the kidney is followed by a subsidence of acute manifestations, and absorption and proliferative organization of the exudate. Many of the glomeruli in this stage show the epithelial and endothelial stimulation and growth seen in a late "acute proliferative glomerulonephritis," or in a subacute glomerulonephritis. True epithelial crescents are occasionally seen. Atrophy of the tubules associated with the more severely injured glomeruli occurs. When the injury is bilateral, a moderate nitrogen retention develops. In the experiments recorded here, this was seen regularly in the tuberculous pigs which had been subjected to renal tuberculin perfusion, and never in the nontuberculous animals similarly treated. With the passage of several months following the intrarenal, or more specifically intraglomerular tuberculin reaction, almost complete restoration to an anatomic normal occurs, at least if very young pigs are used, as was the case in this experiment. As the animals grow and the kidneys develop to normal bulk, the glomeruli increase in size accordingly. The only persisting signs of the former acute inflammation are small hyaline areas which occasionally contain old pyknotic polymorphonuclear leukocytes. These are usually located at the point of vascular attachment of the tuft and capsule. Simultaneously with this anatomic healing, complete functional recovery also occurs, shown by a disappearance of nitrogen retention and return of the blood to normal. The question naturally arises whether or not the artificial production of the nephritis here described has any relation to the spontaneous development of nephritis in man. It will be recalled that the majority of adult human beings in urban groups are sensitive to tuberculin, and therefore carry within themselves the potentiality for the type of injury herein recorded. It is conceivable that during the period of activity of a tuberculous focus, tubercle bacillus protein could be taken up, as by phagocytic cells, from the débris of the lesion and excite changes of an allergic nature at a distant point such as a glomerulus of a kidney. It is unlikely, however, that any such dosage as used in the experiments described could be so absorbed. It is possible, on the other hand, that occasional and scattered glomerular lesions could arise in this way. However, it must be remembered that the tuberculin reaction is only one of a type. A considerable number of analogous forms of sensitization exist. While such reactions are in general protective against

the spread of the infection concerned, the act of protection itself may be destructive to the local tissue, and no initial distinction is made in the reaction to live bacteria or their dead specific protein. These facts, together with the experiments reported here, justify further work in this field.

AUTHORS' SUMMARY.

THE PHAGOCYTIC ACTIVITY OF THE VASCULAR ENDOTHELIUM OF GRANULATION TISSUE. F. A. MCJUNKIN, Am. J. Path. **4**:587, 1928.

Carbon is present in the vessel walls of granulation tissue after india ink has been injected intravenously and also to some extent at the site of subcutaneous injections of ink. The presence of this particulate matter within the cytoplasm of the endothelial cells can be explained only as a process of active phagocytosis. The phagocytic activity of these endothelial cells is identical with that of the Kupffer cells of the liver which have a comparable amount of cytoplasm.

AUTHOR'S SUMMARY.

THE EFFECT OF FEEDING POTASSIUM IODIDE ON THE PROLIFERATIVE ACTIVITY OF THE THYROID GLAND IN GUINEA-PIGS. JACOB RABINOVITCH, Am. J. Path. **4**:601, 1928.

A quantitative estimation of the proliferative activity of the thyroid gland has been carried out, by counting the number of mitoses present in the normal gland as well as in the glands of guinea-pigs fed with various quantities of potassium iodide during different periods of time. The number of mitoses found in thyroids obtained from animals fed with potassium iodide within the first three weeks after the beginning of the feeding is by far greater than that observed in the controls. The increase in the number of mitoses is only slight during the first ten days. It becomes very pronouced between the fifteenth and the twentieth day of the iodide feeding, and lessens again when feeding is continued for thirty days. Within the range of quantities of the substance used by us, larger doses of potassium iodide call forth greater proliferative activities of the thyroid epithelium than smaller doses. At the same time the colloid becomes slightly softer and the phagocytic activity within the colloid increases after potassium iodide has been fed for fifteen to twenty days; the epithelium, however, changes very little in height. At the end of the thirtieth day, the acini enlarge and become irregular, the colloid becomes watery and the epithelium flattens out; the number of phagocytes also diminishes. We attribute the flattening of the acinus epithelium, which is observed in the fifth week, to the pressure exerted on the epithelium by the increase in volume in the colloid resulting from the absorption of water at this period.

AUTHOR'S SUMMARY.

CONGENITAL ATRESIA OF AORTIC RING. N. PHILPOTT, Ann. Int. Med. **2**:422, 1928.

Philpott describes the autopsy observations in the case of an infant who lived sixty-two hours after birth. On the day after delivery, cyanosis appeared and deepened progressively until death occurred. The administration of oxygen was without effect. There was no mitral opening. A direct communication between left and right auricles was established by means of a widely patent foramen ovale. There was complete atresia of the aortic ring. The ductus arteriosus was widely patent.

WALTER M. SIMPSON.

RELAPSING FEBRILE NODULAR NONSUPPURATIVE PANNICULITIS. H. A. CHRISTIAN, Arch. Int. Med. **42**:338, 1928.

A case is reported in which there were irregularly distributed inflammatory nodules in the subcutaneous fat. Ten such attacks occurred in nine years. They were associated with fever up to 104 F., malaise, nausea and vomiting. His-

tologic study of the tissues showed edema and some atrophy of the subcutaneous fat, and infiltration largely by mononuclear types of cells, with occasional giant cells. Bacterial cultures of the affected tissues were negative. As the process healed, there was depression of the skin into the hollowed out places left by atrophy of the fat.

H. R. FISHBACK.

PANCREATOGENOUS FATTY DIARRHEA. T. E. H. THAYSEN, Arch. Int. Med. **42**: 352, 1928.

The case presented is one of fatty diarrhea of five years' duration, associated with chronic diffuse pancreatitis and stone in the pancreatic ducts. Azotorrhea was present to a marked degree, but not glycosuria. Previous workers have suggested the hypothesis that the pancreas passes an internal secretion into the blood, which is active in fat and protein metabolism.

H. R. FISHBACK.

RELATIVE LYMPHOCYTOSIS IN HYPERTHYROIDISM. V. MENKIN, Arch. Int. Med. **42**:419, 1928.

In 100 cases of hyperthyroidism there was a relative lymphocytosis in 67 per cent. Of the group with exophthalmos, there was relative lymphocytosis in 80 per cent, and in these thyroidectomy restored the normal differential formula. This return to normal did not always occur in the patients with lymphocytosis and without exophthalmos.

H. R. FISHBACK.

CHANGES IN THE FUNDUS OCULI AS AN INDEX TO ARTERIAL DISEASE. S. A. AGATSTON, Arch. Int. Med. **42**:455, 1928.

Sclerosis of the arterioles of the kidney and brain cannot exist without a similar condition of the retinal arteries, and normal retinal vessels definitely exclude interstitial nephritis of the sclerotic type. Cases of nephritis on a basis of infection with considerable albuminuria may show a normal fundus or a neuroretinitis with little or no retinal vessel change. The extent of sclerosis of the retinal arteries is generally proportional to the changes in the kidneys, the height of the blood pressure and the nonprotein nitrogen of the blood. Patients with incipient hypertension may suffer from spasmodic contraction of arteries giving temporary blindness, angina or cerebral symptoms. Patients with vascular disease without hypertension are especially liable to thrombosis. Moderate changes of the retinal vessels recognized by the ophthalmoscope may not be found by microscopic study.

H. R. FISHBACK.

SCLERODERMA AND CALCINOSIS. R. H. DURHAM, Arch. Int. Med. **42**:467, 1928.

A review is given of scleroderma and calcinosis as to case reports, theories of pathogenesis and incidence. It is suggested that the two conditions together constitute a distinct pathologic entity.

H. R. FISHBACK.

Pathologic Chemistry and Physics

CHEMICAL CONTRASTS BETWEEN COLLAGENOUS AND RETICULAR CONNECTIVE TISSUE. NATHAN CHANDLER FOOT, Am. J. Path. **4**:525, 1928.

In the connective tissue framework of the spleen in man, isolated by means of tryptic digestion, there are three main groups of fibrous substances: "collagen," elastin and reticulin. The first may be completely extracted by boiling water, leaving the white fibrous tissue incapable of taking specific collagen stains. The extract may be coagulated on slides by means of gentle heat and fixation in Zenker's fluid and a substance will be recovered that gives all the characteristic staining reactions of collagen and many of the precipitation reactions of gelatin. The elastin resists boiling water, and weak acid or alkaline solution. It may be digested

with pepsin and hydrochloric acid, 0.3 per cent. (This was not mentioned in the body of the paper; it is given for the sake of completeness.) The reticulin is composed of a mixture of at least three groups of substances: an alcohol-soluble group (lipins, largely impure lecithin), an alcohol-insoluble group made up of an argyrophil material that may come down as threads and a silver neutral background of amorphous matter that forms the bulk of the fraction; this may be digested fibers. After extracting these substances from the splenic framework, if alkali-digestion has not been carried too far, pale fibers that stain neither with silver nor fuchsin remain; these may be stained with eosin, picric acid or phosphotungstic acid hematoxylin; rose, yellow and reddish, respectively ("true collagen?"). The staining reactions of the substance extracted with boiling water and those of commercial gelatin are not the same. Using the technic herein described, jellies are seldom produced. The argyrophil matter in the reticulin is best demonstrated after exposure to certain oxidizing agents, while reducing agents apparently inhibit impregnation. There is a possibility that the water-soluble "collagen" and the alkali-soluble "reticulin" constitute stiffening, strengthening or protective substances for the fibers of the connective tissue; they impregnate these evenly and may be extracted without destroying the fibers. The assumption that reticulin may be hydrolyzed to collagen is not unwarranted.

AUTHOR'S SUMMARY.

STUDIES OF THE INHIBITORY ACTION OF AN EXTRACT OF PANCREAS UPON GLYCOLYSIS. E. RONZONI, J. GLASER and D. P. BARR, J. Biol. Chem. **80**: 309 and 331, 1928.

A simplied method is described for extracting from pancreas the substance which has the property of inhibiting the normal course of glycolysis in muscle. The substance is not insulin. Its action appears to be directed toward a retardation of the formation of lactacidogen (hexose phosphate). Since the extract appears to have no effect on the glycolysis of cancer tissue, it is concluded that the mechanism of sugar breakdown in such tissue is different from that normally occurring in muscle.

ARTHUR LOCKE.

SEASONAL VARIATIONS IN THE IODINE AND THYROXINE CONTENT OF THE THYROID GLAND. E. C. KENDALL and D. G. SIMONSEN, J. Biol. Chem. **80**: 357, 1928.

The iodine content of the thyroid gland varies with the seasons, reaching a maximum during the warm weather of July and August and a minimum during the colder months of January and February. There appears to be a parallel but considerably less marked fluctuation in the thyroxin content of the gland. The iodine present as thyroxin represents less than 14 per cent of the total amount available. "It is impossible to explain the physiological activity of fresh or desiccated thyroid by the thyroxin which can be separated in crystalline form. Thyroxin appears to be an intermediate form of the active constituent of the thyroid."

ARTHUR LOCKE.

DISTRIBUTION OF UNSATURATED FATTY ACIDS IN TISSUES. W. R. BLOOR, J. Biol. Chem. **80**:443, 1928.

The phospholipid concentration of an organic tissue would appear to be a direct index of its functional activity. It maintains a fair constancy throughout the growth of the organ (beef), reaches a maximum in the cerebral tissues and descending values in the liver, pancreas, kidney and lung, respectively. The phospholipins of these organs are constituted of approximately equal parts of lecithin and cephalin, suggesting the existence of an equimolecular equilibrium or combination between the two substances.

ARTHUR LOCKE.

THE UROBILIN CONTENT OF NORMAL HUMAN BLOOD. M. A. BLANKENHORN, J. Biol. Chem. **80**:477, 1928.

Urobilin zinc acetate has the remarkable and specific property of becoming strongly fluorescent on proper illumination. The property has been made the basis of the Schlesinger test for the presence of urobilin in biologic material and of the Lutz test for the presence of zinc. The Schlesinger test permits the detection of concentrations of urobilin as minute as 0.000,048 mg. per cubic centimeter, but can be used to advantage only when the solutions to be examined are perfectly clear. The illumination of cloudy solutions produces a Tyndallization, which tends to obscure and nullify the looked-for fluorescence. Blankenhorn's procedure is to permit from 2 to 3 cc. of the specimen (clear blood serum) to become coagulated on shaking with 0.5 Gm. of powdered zinc acetate. After further shaking with 3 volumes of absolute alcohol, the well stoppered specimen is held in the icebox for twenty-four hours and then strongly centrifugated. The fluorescent capacity of the clear supernatant fluid is compared in a specially constructed chamber with that of a known dilution of acriflavine. (The use of a light filter, as employed by Lutz [*J. Indust. Hyg.* **7**:273, 1925] to remove that part of the spectrum responsible for the Tyndallization, while retaining the part essential for the production of fluorescence, appears not to have been attempted.) The urobilin content of normal human blood, as indicated by an analysis of 128 varied specimens, appears to be approximately 0.0028 mg. per cubic centimeter.

ARTHUR LOCKE.

THE ACID-BASE COMPOSITION OF GASTRIC SECRETIONS, PANCREATIC JUICE AND BILE. JAMES L. GAMBLE and MONROE A. MCIVER, J. Exper. Med. **48**: 837 and 849, 1928.

The chief inorganic factors in secretions obtained from isolated pouches constructed in the fundus and in the pyloric antrum of the cat's stomach were found to be chloride ion and fixed base. In a series of samples obtained from the fundic pouch, chloride ion was approximately stationary at 165 cc. tenth-normal per hundred cubic centimeters. During digestion of food in the stomach, secretions from the pouch contained fixed base in amounts varying considerably from an average of 47 cc. tenth-normal per hundred cubic centimeters. Material allowed to remain in the pouch after the completion of the digestion of food in the stomach showed an increasing content of fixed base, to as much as 140 cc. tenth-normal per hundred cubic centimeters. A stationary total ionic content of secretions of the fundus is thus seen to be sustained by the chloride ion concentration, and changes in hydrogen ion concentration to be caused by variation of fixed base. The differing amounts of fixed base found are regarded as probably due to admixture of a mucous secretion with the juice from the fundic glands. The alkaline secretion taken from a pyloric pouch contained fixed base in excess of chloride ion. Variation of fixed base in the secretions from the fundic pouch were found to be referable to change in sodium content, the smaller factor, potassium, remaining approximately constant at about the value found in material from the pyloric pouch. This suggests that the mucous secretion of the fundus has the same composition as that produced by the pyloric antrum. These data serve to explain the extensive withdrawal of fixed base, as well as of chloride ion, from the blood plasma in the presence of circumstances causing a continued loss of stomach secretions.

Pancreatic juice contains fixed base at approximately the concentration found in the blood plasma. Chloride ion is present in concentrations varying from one-fourth to one-half the fixed base value and the remainder of the acid equivalence is composed of bicarbonate ion. Fixed base being a nearly stationary factor, variation of bicarbonate and thereby of alkalinity is referable to change in the concentration of chloride ion. In bile, as delivered by the liver, both the fixed base and the chloride ion values correspond closely with their respective concentrations in blood plasma. In gallbladder bile, however, the concentration of fixed base is, roughly, double that in hepatic duct bile, and chloride ion has been almost entirely

removed. From these data, it may be inferred that loss of digestive secretions entering the duodenum will, in the absence of replacement of the materials contained, cause dehydration of the blood plasma and reduction of the plasma bicarbonate.

AUTHORS' SUMMARY.

CHANGES IN FLUIDS OF BODY FROM LOSS OF THE EXTERNAL SECRETION OF THE PANCREAS. JAMES L. GAMBLE and MONROE A. MCIVER, J. Exper. Med. **48**:859, 1928.

The following explanation of the effects of continued loss of the external secretion of the pancreas may be offered. The underlying event is a steadily increasing deficit of sodium and of chloride ion due to the large requirement for these electrolytes in the construction of pancreatic juice. In consequence there is continued loss of water, chiefly from the body fluids in which sodium and chloride ion are large factors of total ionic content, viz., interstitial fluids and the blood plasma. During about two thirds of the survival period, the volume and composition of the blood plasma remain approximately normal, the losses of water, sodium and chloride ion being replaced at the expense of interstitial fluids. Reduction of the volume of these fluids is indicated by loss of body weight, beginning directly after establishment of the pancreatic fistula. Ultimately, reduction of the volume of the plasma begins and, as it progresses, serious symptoms develop and death occurs, unless water, sodium and chloride ion are abundantly replaced. Owing to the relatively greater loss of sodium than of chloride ion in pancreatic juice, reduction of bicarbonate ion concentration in the plasma tends to occur. The death of the organism may be simply and reasonably explained as the result of progressive impairment of the function of the blood by the physical changes, dehydration and acidosis, produced in the plasma by the continued loss of sodium and of chloride ion in the pancreatic juice.

AUTHORS' SUMMARY.

ESTIMATIONS OF THE HYDROGEN ION CONCENTRATION OF THE URINE. F. MAINZER, Klin. Wchnschr. **8**:109, 1929.

Urine taken with the exclusion of air at 38 C. is best suited for the determination of the hydrogen ion concentration. This method is practicable with single specimens, but difficult with collections of many hours. For collected samples, the measurement of the hydrogen ion concentration with a carbon dioxide tension of 40 mm. mercury at room temperature is satisfactory. On this basis is the advantage of the method mentioned over measurements disregarding the carbon dioxide tension.

ARTHUR'S SUMMARY.

Microbiology and Parasitology

RICKETTSIA IN MOSQUITOES (AEDES AEGYPTI) INFECTED WITH THE VIRUS OF DENGUE FEVER. ANDREW WATSON SELLARDS and JOSEPH F. SILER, Am. J. Trop. Med. **8**:279, 1928.

Masses of *Rickettsia* were frequently found in mosquitoes (*Aedes aegypti*) which were known to be infected with the virus of dengue fever. No *Rickettsia* was found in control mosquitoes.

A contaminating protozoön resembling developmental forms of *Lankesteria culicis* occurred in some of the control mosquitoes. This contaminating organism can, under certain conditions, offer some confusion in the study of *Rickettsia* in smears.

AUTHORS' SUMMARY.

THE FLAGELLATED PROTOZOA OF THE INTESTINE. KENNETH N. LYNCH, Am. J. Trop. Med. **8**:345, 1928.

There was no evidence that diarrhea was related in any way to the presence of *Trichomonas hominis* or *Chilomastix mesnili*. There was no evidence that constipation was related to the presence of *Trichonomas*, but constipation was a

common habit of the persons harboring *Chilomastix*. Among 240 unwell persons harboring intestinal flagellates, there was no case of arthritis deformans. The presence of *Trichomonas hominis* appears to be more related to rural life and to warm climates, while the presence of *Chilomastix mesnili* appears to be more related to city life but relatively unrelated to climate. Infestation with *Giardia intestinalis* was more frequent in early life; *Chilomastix mesnili*, in later life; while the age of the person had apparently no relation to the presence of *Trichomonas hominis*. A state of lowered acidity of the stomach appeared to be favorable to the presence of *Chilomastix* but of no relation to *Trichomonas*. The observation is made that a clinical diagnosis of chronic cholecystitis was recorded in about twice as many of the carriers of *Trichomonas* and *Chilomastix* as in the flagellate-free.

AUTHOR'S SUMMARY.

THE STREPTOCOCCUS, AN ALLY OF SMALLPOX. J. G. CUMMING, Contributions to Medical Science Dedicated to A. S. Warthin, Ann Arbor, George Wahr, 1927, p. 283.

In a series of twenty successive cases in patients with smallpox admitted to the Isolation Hospital, Washington, D. C., Cumming found eight persons who were tonsillar carriers of hemolytic streptococci. In the group of twenty patients, there were six deaths, all among the streptococcus carirers. In all the fatal cases, hemolytic streptococci were isolated, not only from the throat, but also from the blood stream within forty-eight hours before death or from the heart's blood immediately after death, or from both sources.

Cumming concludes that in fatal cases of smallpox the immediate cause of death is a septicemia caused by hemolytic streptococcus, in which the hemolytic streptococcus is activated to its maximum virulence by the smallpox virus.

WALTER M. SIMPSON.

THE REACTION OF THE OMENTUM TO GERM SUBSTANCE. W. T. VAUGHAN, Contributions to Medical Science Dedicated to A. S. Warthin, Ann Arbor, George Wahr, 1927, p. 503.

In a study of the reaction of the omentum to foreign material implanted within the peritoneal cavity, Vaughan inoculated each of a series of guinea-pigs with 10 mg. of dead tubercle bacillus germ substance in 1 cc. of physiologic solution of sodium chloride. Within twenty-four hours, the omentum had gathered up practically all of the material, and it was found closely adherent to the omental surface. In its reaction, the omentum often becomes adherent to adjacent structures. In control animals inoculated with Tobler bacillus germ substance, bone ash, lycopodium spores and animal charcoal, the injected material was invariably found attached only to the omentum.

Microscopically, in animals killed twenty-four hours after inoculation, the tubercle bacillus germ substance is found attached to the surface by fibrinous exudate or incorporated in the tissues just beneath the surface. The foreign material is surrounded by a heavy accumulation of leukocytes and wandering cells with large, clear, pale-staining nuclei, and a few lymphocytes.

At the end of a week, the clumps of germ substance are surrounded by whorls of rather dense connective tissue fibrils. The wandering cells and polymorphonuclear leukocytes persist in great numbers. The larger the foreign body mass the less extensive is the proliferation of the connective tissue, and actual suppuration occurs.

After thirteen days, the small masses of foreign bodies have been practically entirely removed, and the site of each mass is replaced by a dense connective tissue whorl. The wandering cells now clearly predominate over the leukocytes. The connective tissue reaction to the larger masses is well advanced.

After thirty-three days, the reparative changes are quantitatively greater. There was evidence that other organs with peritoneal covering aid in the removal of the particulate matter.

The reaction of the omentum to foreign body substance does not differ essentially from such reaction in other tissues the functions of which are not so clearly protective. The tendency toward encapsulation may be somewhat more pronounced, thus accounting for the occasional development of omental cysts, not embryonic in origin.

WALTER M. SIMPSON.

VIRUS III ENCEPHALITIS. THOMAS M. RIVERS and FRED W. STEWART, J. Exper. Med. **48**:603, 1928.

Virus III, an active, filtrable agent indigenous to rabbits, under experimental conditions, produces, in addition to lesions in the cornea, skin and tests, an encephalitis which is at times fairly similar to that induced by herpetic virus. Virus III and herpetic virus, however, are not immunologically related.

AUTHORS' SUMMARY.

CHEMOTHERAPY IN EXPERIMENTAL OROYA INFECTION. HIDEYO NOGUCHI, J. Exper. Med. **48**:619, 1928.

The therapeutic effect of several antiparasitic chemicals on experimental verruga peruana is described. The drugs were administered by intravenous injection according as the nodules were already developed to an approximate maximum, or were still in the active period of growth. The effect of the drugs was different under the two circumstances of their administration. When they were given after the maturity of the nodules, they hastened the regressive process, but when given during active growth of the lesions, no action was detected. *Bartonella bacilliformis* in culture is acted on injuriously by a number of the chemicals employed in the therapeutic tests, the most active being formaldehyde and neutroflavine.

AUTHOR'S SUMMARY.

A PARATYPHOID INFECTION IN GUINEA-PIGS. JOHN B. NELSON, J. Exper. Med. **48**:647, 1928.

A group of 105 breeders and 36 unweaned guinea-pigs was tested to determine the extent of specific fecal excretion and the proportion of serum reactors in a population naturally infected with two types of *B. paratyphi*. The second more recent type of organisms was isolated from the feces of three breeders and three young guinea-pigs. No carriers of the first type were detected. Eighty-six per cent of the breeders and 72 per cent of the unweaned guinea-pigs agglutinated the second type of *B. paratyphi* in dilutions ranging from 1:10 through 1:640. Thirteen per cent of the breeders and 28 per cent of the unweaned young guinea-pigs agglutinated the initial type. There was a cross or double agglutination in most instances. The serologic observations roughly reflected the distribution of the two types as indicated by the mortality rate of the population at large. Fifty breeders selected on the basis of agglutination and fecal examination and therefore supposedly free from infection were segregated and kept under close observation. Both types of *B. paratyphi* subsequently appeared in the group. During this time, carriers were discovered by others in the department among the stock guinea-pigs used for other experiments in that cultures of the entire spleen were positive in perhaps from 5 to 10 per cent of the guinea-pigs so used.

AUTHOR'S SUMMARY.

EXPERIMENTAL PNEUMONIA IN GUINEA-PIGS. JULIA T. PARKER and ALWIN M. PAPPENHEIMER, J. Exper. Med. **48**:695, 1928.

Anaerobic autolysates of pneumococci, prepared according to the method described, are highly toxic for guinea-pigs when injected intratracheally in a dosage of 0.2 cc. Death occurs either within a few hours (36 per cent) or within three days. In the early deaths there is intense hemorrhagic edema of the lungs with beginning inflammatory reaction; in animals surviving for eighteen hours or longer extensive areas of pneumonia are produced. The intratracheal injection of

virulent living pneumococci is followed by transient slight lesion with recovery, or by later death from septicemia without pneumonic lesions. The addition of a sublethal dose of toxic autolysate to living pneumococci alters the reaction of the animal, so that an extensive pneumonia develops associated with unrestrained multiplication of the organism.

AUTHORS' SUMMARY.

BACTERIOPHAGE OF PSYCHROPHILIC ORGANISM. A. LAWRENCE ELDER and FRED W. TANNER, J. Infect. Dis. **43**:403, 1928.

A bacteriophage active for a psychrophil has been obtained. The specificity of the bacteriophage was fairly pronounced, as shown by the fact that it was not readily active on any of fifty-nine other psychrophils studied. Macroscopic observations of the lytic action confirmed all the usual characteristics of bacteriophagy.

AUTHORS' SUMMARY.

MUCOSUS ORGANISM FROM SUPPURATIVE LESIONS OF RAT ON DIET DEFICIENT IN VITAMIN A. WILLIAM L. BRADFORD, J. Infect. Dis. **43**:407, 1928.

By aerobic cultures from the suppurative terminal lesions in the white rat on a diet deficient in vitamin A, an encapsulated bacillus of the mucosus encapsulated group has been isolated in about one-half the cases. From fifty rats on A-deficient diet, representing three different stocks, twenty-one strains were isolated. In thirteen normal rats three rats on B-deficient diets and nine rats on D-deficient diets, all without obvious infections, cultures from the ears and nose failed to reveal the organism. It was recovered once in a series of ten wild (brown) rats from the nose. Although obviously more frequently present in the suppurative conditions of the animal on an A-deficient diet, it is probably a secondary invader of mucosa of the respiratory tract, made suitable for its invasion by the dietary deficiency. The organism has been found to be suitable for use in a study of the relationship between vitamin deficiencies in diet and resistance to infection which will be reported.

AUTHOR'S SUMMARY.

ETIOLOGY OF DERMATITIS OF EXPERIMENTAL PELLAGRA IN RATS. W. D. SALMON, I. M. HAYS and N. R. GUERRANT, J. Infect. Dis. **43**:426, 1928.

The external symptoms of experimental pellagra of the rat are alopecia, dermatitis, stomatitis, ophthalmia and arthritis, and usually cachexia. The internal lesions of advanced cases are hemorrhagic gastro-enteritis, atrophy of the spleen, fatty infiltration or degeneration of the liver, cloudy swelling of the kidneys and often cystitis. There is a relation between the character of the diet and the occurrence of the syndrome. A mild dermatitis has been found among mature rats receiving a diet which is adequate for excellent growth and reproduction. The severe form has been produced only on restricted diets.

A gram-positive coccus has been found constantly associated with the disease. This organism has been isolated (often in pure culture) from skin lesions, arthritic lesions, parenchymatous organs and walls of the intestines of pellagrous rats. With two exceptions the organism has not been obtained from the blood stream. The organism has been fed to rats and the characteristic lesions, from which the organism has been recovered, have been produced.

Concentrates of a protective principle (P-P factor) which cure or prevent the occurrence of the disease have been prepared from *Pueraria thunbergiana*. A relatively low concentration of these preparations in nutrient broth inhibits the growth of the causative organism.

AUTHORS' SUMMARY.

OBSERVATIONS ON SNUFFLES IN RABBITS. F. RENE VAN DE CARR and KATHLEEN J. KILGARIFF, J. Infect. Dis. **43**:442, 1928.

A systemic response of the rabbit to infection of the respiratory tract with *B. lepisepticum* and *B. bronchisepticus* was demonstrated by serologic and allergic

reactions. The serologic and allergic reactions correlate with the bacteriologic observations to a high degree: the allergic reactions to a higher degree than the serologic reactions. The allergic reactions were apparently specific. They may prove to be a simple, more direct method of detecting infected or immune rabbits.

B. lepisepticum or *B. bronchisepticus*, or both, were demonstrated in every case of clinical snuffles in this study. *B. lepisepticum* or *B. bronchisepticus*, or both together, may be responsible for snuffles in rabbits. In manifestations of acute snuffles, such as bronchopneumonia, pleurisy, pericarditis and septicemia, *B. lepisepticum* alone was demonstrable.

A 2 per cent solution of silver nitrate was found to be useful in separating the carriers from the uninfected animals in a group of clinically healthy rabbits.

AUTHORS' SUMMARY.

THE PROTEUS HEMOLYSIN. JOHN F. NORTON, ELIZABETH VERDER and CATHERINE RIDGWAY, J. Infect. Dis. **43**:458, 1928.

The production of an agent which is lytic for red blood cells is characteristic of the proteus group of bacteria. The agent is heat labile, is formed during the first few hours of growth in a culture, is rapidly destroyed by phenol disinfectants, is absorbed by a Berkefeld N filter and is neutralized by both homologous and heterologous antiserums.

AUTHORS' SUMMARY.

THERMOPHILIC BACTERIA IN CANNED FOODS. MYRTLE SHAW, J. Infect. Dis. **43**:461, 1928.

Twenty-three strains of obligate thermophils were isolated from sixty-five cans of fruits and vegetables, and classified in two groups. Twenty-one were so nearly identical as to be considered of the same species, all but three of which were obtained from corn and peas which were "flat sours," as indicated by the hydrogen ion concentration. This would indicate a fairly homogeneous group responsible for this type of spoilage. Two cultures were obtained from swelled cans of pumpkin. These cultures seemed unlike the majority of those in the group of twenty and unlike any previously described in the literature. They were considered a new species and named *Bacillus pepo*.

Facultative thermophils were not found.

Two variations of standard technic were devised and used in the determination of reactions which, with the use of standard methods, were weak or indefinite. These were variations in the use of potato slants and starch agar plates.

AUTHOR'S SUMMARY.

RELATIONSHIP BETWEEN CHRONIC INTESTINAL STASIS AND ANEMIA. PAUL R. CANNON, J. Infect. Dis. **43**:480, 1928.

Chronic intestinal obstruction with resulting stasis in the ileum has been produced in forty-five albino rats. Diets favoring the development of proteolytic bacteria have been fed to such animals in an attempt to determine whether or not hemolytic substances may be formed and absorbed and thus lead to an anemia.

In most instances, no significant anemia occurred; whenever it did develop under these conditions, it was possible to demonstrate evidences of a concomitant acute infection.

Chronic ileal stasis in the albino rat, with a proteolytic intestinal flora, may be present for several weeks with no significant increase in blood destruction, provided an intercurrent infection does not supervene. Consequently, in experimental animals, one cannot conclude that an anemia is due to the absorption of hemolytic substances from the lumen of the bowel until all evidences of acute infection have been eliminated by bacteriologic and histologic procedures.

AUTHOR'S SUMMARY.

NEISSERIA SUBFLAVA (BERGEY) MENINGITIS IN AN INFANT. HARRIET BENSON, ROSE BRENNWASSER and DOROTHY D'ANDREA, J. Infect. Dis. **43**:516, 1928.

This report describes the clinical progress, the bacteriology and the results of the postmortem examination, including an extended account of the alterations in the brain, of a chronic meningitis in a child 7 months of age from whose spinal fluid *N. subflava* was repeatedly isolated. Anatomically, there was a marked internal hydrocephalus and a pressure displacement by a circumscribed abscess of the tissues of the inferior portion of the cerebellum and the upper part of the spinal cord.

The prolonged mild course of the illness continuing for about three months was a notable clinical feature. Gram-negative diplococci observed in stained preparations of spinal fluid commonly are considered to be meningococci. This conclusion cannot be safely made without careful study of the organisms in culture mediums and by serologic tests. Although the members of the flava group as well as *N. catarrhalis* and *N. sicca* have little pathogenicity in human hosts, they seem to be able to produce disease in susceptible persons.

AUTHORS' SUMMARY.

SPONTANEOUS TUBERCULOSIS IN RABBITS. MALCOLM J. HARKINS and ELI R. SALEEBY, J. Infect. Dis. **43**:554, 1928.

Two cases of tuberculosis in rabbits are described. Besides the rarity of the disease in rabbits the most interesting feature was the atypical gross and histologic appearance of the nodules in one of the animals. The distribution on the serous layers and the atypical cells suggested a neoplasm of the endothelioma type. Continued passage through other rabbits resulted in more nearly typical nodules. Tubercle bacilli were demonstrated in both cases.

AUTHORS' SUMMARY.

GLUCOSE INHIBITION OF EXTRACELLULAR TOXIN-PRODUCING ENZYMES OF CLOSTRIDIUM BOTULINUM. C. N. STARK, J. M. SHERMAN and PAULINE STARK, J. Infect. Dis. **43**:566, 1928.

It is shown that when *Clostridium botulinum* is grown in a medium containing dextrose there results an inhibition of the production of the enzymes responsible for the extracellular formation of toxin. It is also noted that the toxin produced in dextrose broth is more stable than that formed in the same medium without the carbohydrate.

AUTHORS' SUMMARY.

DESTRUCTION OF DIPHTHERIA TOXIN BY BACTERIA. C. N. STARK, J. M. SHERMAN and PAULINE STARK, J. Infect. Dis. **43**:569, 1928.

It has been shown that several bacteria have the power to destroy diphtheria toxin when grown in its presence. The data reported show this to be true of *Bacterium coli, Bacillus cereus, Proteus vulgaris, Pseudomonas pyocyaneus* and *Clostridium sporogenes.* Especially active destruction was caused by *Clostridium sporogenes.*

AUTHORS' SUMMARY.

INCIDENCE OF PFEIFFER'S BACILLUS IN THROATS DURING EPIDEMIC AND INTEREPIDEMIC PERIODS IN CHICAGO. JANET M. BOURN, J. Prev. Med. **2**:441, 1928.

Pfeiffer's bacillus was found with relative frequency in normal throats during interepidemic times. During one period marked by an unusual number of acute infections of the respiratory tract, the incidence of this organism in persons with normal throats was considerably increased. In cultures from the throat in cases of lobar and bronchopneumonia and pulmonary tuberculosis, the Pfeiffer bacillus was found much more frequently than in cultures from normal throats. In persons suffering with colds, sore throats or some mild irritation of the respiratory

mucosa, the incidence of the bacillus was slightly higher than that observed in persons with normal throats. The Pfeiffer bacillus was found in normal throats during interepidemic periods in the same proportion as it was found in association with the diseases of childhood.

AUTHOR'S SUMMARY.

EFFECT OF HEAT-KILLED CULTURES OF S. AERTRYCKE IN MONKEYS AND OTHER ANMIALS. GAIL M. DACK, P. and H. HARMON and IRENE E. JARRA, J. Prev. Med. **2**:461, 1928.

Monkeys, rabbits, dogs and cats fed with heat-killed cultures of *S. aertrycke* failed to show the characteristic gastro-intestinal disturbances present in man in paratyphoid intoxication.

AUTHORS' SUMMARY.

UNSUCCESSFUL ATTEMPT TO PRODUCE SALMONELLA INTOXICATION IN MAN. GAIL M. DACK, WILLIAM E. CARY and PAUL H. HARMON, J. Prev. Med. **2**:479, 1928.

Heat-killed dextrose-broth beef-heart cultures and filtrates of five strains of *Salmonella aertrycke* and four strains of *Salmonella enteritidis*, when fed in large amounts to twenty-four adults on an empty stomach, failed to produce any symptoms, although the same materials produce symptoms and death when injected intravenously into rabbits in amounts of from 0.5 to 2 cc. No agglutinins for homologous strains were present in the serums of these subjects ten days after feeding.

AUTHORS' SUMMARY.

THE CULTURE OF THE SPIROCHETE OF RELAPSING FEVER. MARIO LAPIDARI and HÉLÈNE SPARROW, Arch. Inst. Pasteur de Tunis **17**:191, 1928.

The Ungermann medium in which inactivated rabbit serum with Ringer's solution was tubed in long narrow tubes containing 1 cc. of coagulated egg albumin, the final product being covered with petrolatum, was found by the authors to be the best type of medium for cultures of the spirochetes of relapsing fever. They found that Hartley's tryptic digest broth (horse meat, digested with Cole and Onslow pancreatic extract), substituted for Ringer's solution, improved the medium.

A detailed study of cultures demonstrated the possibility of serial transfer in a large number of cases. It was also observed that the material was at times infectious when no spirochetes could be found. This proof of existence of an invisible infectious agent, coupled with the statistical observations on the latent periods in the test tube cultures wherein potential spirochetes existed, leads the authors to suggest that they may have observed in vitro the existence of cyclic stages in the spirochete, part of which are invisible, such as Nicolle believes to occur in man in relapsing fever.

M. S. MARSHALL.

MYCETOMA OF THE THIGH DUE TO A TRICHOMYCETES, NOCARDIA NICOLLEI N. SP. P. DELANOË, Arch. Inst. Pasteur de Tunis **17**:257, 1928.

A Moroccan adult was infected on the thigh with this fungus, considered to be a new species. The lesion, when first seen, dated back eleven years, when an inguinal adenitis developed which later spread. The right thigh showed marked tumefaction, the tissues being edematous with a dense infiltration. Covering a large area below the inguinal region were numerous hemispherical or elongated raised nodules of considerable size, giving the impression of cold abscesses. The contents of these lesions when first opened were serous, then bloody and serous and finally frankly bloody. The fluid contained numerous yellowish granules, varying in size. There were unquestionably metastases in other parts of the body.

A detailed description of the culture isolated from the granules and a photograph of the case are included in the article.

M. S. MARSHALL.

THE HEAT TREATMENT OF STREPTOCOCCUS INFECTIONS. B. MENDEL, F. STRELITZ and M. BAUCH, Klin. Wchnschr. **7**:1899, 1928.

In vitro experiments with three strains of streptococci demonstrated that temperatures between 40.5 C. for twenty-four hours and 48 C. for fifteen minutes altered the organisms so that ten times the lethal dose was not regularly fatal in mice, and only with 100 times the lethal doses were all the animals killed. Rats into which injections of lethal doses of streptococci were made and kept at 41.5 C. for eight hours lived; control animals died regularly. Treatment with heat for streptococcus infections is recommended on the basis of these results.

E. F. HIRSCH.

AGRANULOCYTOSIS. W. SCHULTZ, München. med. Wchnschr. **75**:1667, 1928.

Schultz summarizes the reports and new features recorded since his original publication. He restates the symptoms, the changes in tissues, the prognosis and the therapy. A specific causal agent has not been established.

E. F. HIRSCH.

PATHOLOGIC ANATOMY OF TUBERCULOSIS OF THE MESENTERIC GLANDS. M. BOCK, Ztschr. f. Tuberk. **52**:30, 1928.

In all cases of chronic tuberculosis of the mesenteric glands, there are inflammatory changes in the capsule of the gland, in the surrounding tissue and in the peritoneum, which is in direct contact with the glands. These alterations are partly tuberculous and partly nonspecific. The inflammatory alterations of the peritoneum and of the mesentery cause pain on pressure, diarrhea, constipation and increased mucous secretion.

MAX PINNER.

THE BLOOD PICTURE IN TUBERCULOSIS IN CHILDHOOD. L. HINDERSIN, Ztschr. f. Tuberk. **52**:34, 1928.

Hematologic alterations occur on small stimuli. The blood picture is therefore, if critically applied, of great significance in the therapeutic indication, but its prognostic significance extends only over short periods of time.

MAX PINNER.

HOHN'S METHOD OF ISOLATING TUBERCLE BACILLI. W. ROLOFF, Ztschr. f. Tuberk. **52**:153, 1928.

By Hohn's method it was possible to demonstrate the presence of tubercle bacilli in the sputum in twenty microscopically negative specimens. This method is nearly as reliable as animal inoculation and is much simpler.

MAX PINNER.

Immunology

THE DETERMINATION OF RATE OF HEMOLYSIS BY THE MEASUREMENT OF LIGHT TRANSMISSION. H. D. KESTEN and T. F. ZUCKER, Am. J. Physiol. **87**:263, 1928.

By the use of a photo-electric cell, calibrated for its purpose by means of blood suspensions of known hemolysis, the writers devised a means of determining the rate of hemolysis. Certain spontaneous changes in light transmission through dilute blood suspensions are discussed.

H. E. EGGERS.

SAPONIN HEMOLYSIS OF HUMAN BLOOD. H. D. KESTEN and T. F. ZUCKER, Am. J. Physiol. **87**:274, 1928.

By means of the photo-electric method previously described, the writers studied the rate of saponin hemolysis of normal blood, as well as of blood from

anemic patients. The latter hemolyzed more slowly than the normal. The effects of temperature, red cell concentration and saponin concentration are discussed.

H. E. EGGERS.

SAPONIN HEMOLYSIS OF RETICULOCYTE-CONTAINING BLOOD. T. F. ZUCKER and H. D. KESTEN, Am. J. Physiol. **87**:280, 1928.

In rabbits in which there had been induced marked secondary anemia by repeated bleeding, the saponin hemolysis rate was determined by the writers' photo-electric method, for the entire blood, and for the separate portions with high and low reticulocyte content. In reticulocyte-containing blood the hemolysis rate was found to be at first faster, then slower, than that of normal blood. Rapid blood regeneration was not found to result in the production of even a relatively homogenous group of more resistant cells.

H. E. EGGERS.

AN IMMUNE PHENOMENON IN EXPERIMENTAL RELAPSING FEVER. HENRY EDMUND MELENEY, J. Exper. Med. **48**:805, 1928.

In five splenectomized squirrels and chipmunks that were reinoculated with a strain of *Spironema recurrentis* which had previously been present in their blood, the first attack was entirely suppressed because the animals were immune to the strain of spirochetes inoculated; but after the interval which usually occurred between attacks, a relapse ensued, in which the strain of spirochetes present in the blood was different from the strain inoculated.

AUTHOR'S SUMMARY.

FLAGELLAR AND SOMATIC AGGLUTINATION. JOHN B. NELSON, J. Exper. Med. **48**:811 and 825, 1928.

Whole, shaken and heated suspensions of two *Salmonella* species were compared as to agglutinability, absorptive capacity and antigenic properties. The results were in general agreement with the flagellar antigen concept of Smith and Reagh. The removal of flagella by shaking or heating (100 C.) resulted in altered agglutinability manifested by failure to give a floccular reaction with "whole" antiserum. The deflagellated bacteria were able to absorb some flocculating agglutinin from that serum. They were unable, however, to produce flocculating agglutinin on injection in rabbits. Untreated, shaken and heated suspensions of a nonmotile bacterium (*Staphylococcus*) showed no differences with respect to agglutinability or absorptive capacity. Soluble precipitable material was found present in small amount in culture filtrates of the motile bacteria and in greater concentration in filtrates of heated suspensions. The bulk of the soluble material was of somatic origin and was not appreciably increased by the presence of flagella. It was possible, however, to demonstrate soluble material in heated flagella suspensions. The relation of such soluble substances to floccular agglutination and the production of flocculating agglutinin as suggested by Hadley is discussed.

It was shown that flocculating (flagellar) agglutinin and granulating (somatic) agglutinin display certain differences with respect to their removal from sensitized bacteria (*B. paratyphi*). A 5 per cent solution of sodium chloride added to sedimented, sensitized bacteria followed by heating to 60 C. for one hour removed approximately 50 per cent of the combined agglutinin. There was little or no removal of granulating agglutinin either from the sensitized motile bacteria or from a sensitized nonmotile organism (*Staphylococcus*). Evidence was presented that the agglutinin removal was not dependent solely on disintegration of flagella by the conditions of extraction with a subsequent freeing of antibody.

AUTHOR'S SUMMARY.

THE PULMONARY CIRCULATION IN THE GUINEA-PIG DURING ANAPHYLACTIC SHOCK. STEPHEN WENT and CECIL K. DRINKER, J. Exper. Med. **49**:21, 1929.

Sheep serum in doses below 0.3 cc. intravenously produces no pulmonary vasoconstriction in the guinea-pig. Guinea-pigs have consequently been sensitized with this substance and anaphylactic shock produced by doses of 0.1 and 0.2 cc. Pressure in the pulmonary artery has been measured by the method of Drinker and West and recorded photographically in a new and convenient manner. At an early stage in anaphylactic shock the pulmonary arterial pressure falls markedly, and this fall seems to precede the appearance of bronchiolar obstruction. The fall in pulmonary blood pressure in anaphylactic shock is in marked contrast to the rise in pressure resulting from intravenous injection of toxic foreign protein, such as horse serum.

AUTHOR'S SUMMARY.

HYPERSENSITIVENESS TO DIPHTHERIA BACILLI. JAMES M. NEILL and WILLIAM L. FLEMING, J. Exper. Med. **49**:33, 1929.

The paper describes an "immediate" skin reaction to derivatives of the diphtheria bacillus which is shown to be distinct from the "delayed" or "pseudoreaction" commonly seen in Schick tests on adults. The "immediate" reaction was passively transferred to local areas of the skin of other people.

AUTHOR'S SUMMARY.

SENSITIZING PROPERTIES OF THE BACTERIOPHAGE. CLAUS W. JUNGEBLUT and EDWIN W. SCHULTZ, J. Exper. Med. **49**:127, 1929.

Marked specific contractions of the uterine horns of guinea-pigs, actively sensitized, to phage-lysed Flexner bacilli or to colon bacilli, lysed by the same bacteriophage, occurred on testing either series for anaphylaxis with the homologous phage lysates. These reactions, however, were not due to an antigenic function of the bacteriophage itself, because no reaction whatsoever occurred when the same bacteriophage, propagated on the heterologous organisms, was substituted in the anaphylactic test. Specific uterine reactions of marked intensity were obtained in guinea-pigs, actively sensitized to intact or autolyzed Flexner or colon bacilli, respectively, by testing either series for anaphylaxis with homologous, phage-free bacterial antigens. No reaction occurred by testing the uterine strips of animals, sensitized to intact or autolyzed bacilli (either Flexner or coli), for anaphylaxis with homologous phage lysates and, vice versa, there was no contraction of uterine strips sensitized to phage lysates on contact with homologous bacterial autolysates. The observations made in this paper suggest that a new and immunologically distinct antigenic complex arises from the bacterial protein after lysis of the organisms by the bacteriophage.

AUTHOR'S SUMMARY.

RELATIONSHIP BETWEEN A VARIETY OF SACCHAROMYCES CEREVISIAE AND THE TYPE II VARIETY OF DIPLOCOCCUS PNEUMONIAE (PNEUMOCOCCUS). JOHN Y. SUGG and JAMES M. NEILL, J. Exper. Med. **49**:183, 1929.

The paper reports evidence of an immunologic relationship between one variety of *Saccharomyces cerevisiae* and the type II variety of *Diplococcus pneumoniae* (pneumococcus). The most convincing data consisted of the reactions of the type II bacteria with potent antiyeast serum which agglutinated, and protected mice against these pneumococci as well as the average antiserum obtained by immunization of rabbits with type II bacteria themselves. The reactivity of the antiyeast serum is strictly specific to the type II variety of pneumococcus in the sense that it is entirely devoid of antibodies reactive with type I or III. The results of absorption experiments with both the antiyeast (rabbit) serum and the anti-type II (horse) serum were the same as those usually obtained in analogous experiments with immunologically related, but not identical, kinds of bacteria. The

immunologic relationship of the yeast and the type II pneumococcus is apparently based on S-anti-S reactions. It represents an example of heterogenetic specificity which is of particular interest because of the wide genetic separation of the pathogenic schizomycete and the saprophytic ascomycete. Data on the individual irregularity in the yeast-agglutinating capacity of serum from nonimmunized or "normal" rabbits are presented as experimental facts.

AUTHOR'S SUMMARY.

ANAPHYLAXIS WITH THE TYPE-SPECIFIC CARBOHYDRATES OF PNEUMOCOCCUS. OSWALD T. AVERY and WILLIAM S. TILLETT, J. Exper. Med. **49**:251, 1929.

The type-specific carbohydrates (haptens) of pneumococcus types I, II and III, when isolated in protein-free form, are devoid of the property of inducing active anaphylactic sensitization in guinea-pigs. The bacterial carbohydrates of pneumococcus, of which the type II and type III substances are nitrogen-free, produce rapid and fatal anaphylactic shock in guinea-pigs passively sensitized with the precipitating serum of rabbits immunized with pneumococci of the homologous type; the reactions induced are type specific. In contrast to the positive results with immune rabbit serum, there is a complete absence of anaphylactic response to pneumococcus carbohydrate in guinea-pigs passively sensitized with antipneumococcus horse serum.

AUTHOR'S SUMMARY.

CELL ANTIGENS AND INDIVIDUAL SPECIFICITY. KARL LANDSTEINER, J. Immunol. **15**:589, 1928.

Since species are characterized not only by morphologic attributes but by their specific biochemical constitution as well, it seems evident that the somatic and functional development of the organic world was paralleled by a biochemical evolution of the proteins and haptens. One must assume that the two lines of events are linked in some way, although no explanation has been offered as to how such a correlation might be brought about. In this regard it is essential to know whether the small initial steps in evolution are coupled with changes in the proteins. If so, individual and racial protein differences, perhaps too small to be detected by the methods available, ought to be a matter of regular occurrence. In the opposite cases it is conceivable that the transformation of proteins came about discontinuously, contingent on the occurrence of numerous changes or modifications of a special kind in the germinal constitution, perhaps in connection with hapten variations. It is too hazardous, attractive as it would be, to speculate further along these lines. Yet to perceive the problem may not be superfluous and may lead to experimental investigation.

AUTHOR'S SUMMARY.

PREVENTIVE VACCINATION OF THE NEW-BORN AGAINST TUBERCULOSIS BY B. C. G. A. CALMETTE, Brit. J. Tuberc. **22**:161, 1928.

Calmette presents a brief argument and reasons for favoring vaccination of the new-born with B. C. G., and considers Great Britain one of the rare countries in which preventive vaccination does not yet seem to interest public health authorities. From July 1, 1924, to July 1, 1928, 96,000 children have been vaccinated in France with B. C. G., and the method has rapidly spread in many foreign countries, including Belgium, Holland, Norway, Sweden, Poland, Roumania, Russia, Bulgaria, Jugo-Slavia, Greece, Italy and Spain. In French Indo-China, 45,000 children have been vaccinated. Calmette submits himself and his claims to the judgment of the commission set up by the Section of Hygiene of the League of Nations. B. C. G. can be used on the new-born without the slightest fear of producing harm.

H. J. CORPER.

ALLERGY FROM DIPHTHERIA ANATOXIN. A. COMPTON, Brit. M. J. **2**:1175, 1928.

On the third day after the third injection of anatoxin, the patient developed a widespread urticaria.

BLOOD GROUPS AND DISEASES OF THE POPULATION OF SCHLESWIG-HOLSTEIN. M. GUNDEL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **56**:60, 1928.

The blood groups of 2,448 psychopathic patients were determined. A considerable increase of the frequency of group B was noticed among patients with dementia praecox, psychopathia and metasyphilitic diseases, while this group was present in normal or subnormal values among patients with epilepsy, and senile and arteriosclerotic dementia. A definite explanation of the causes could not be offered. The minor increase of group AB and the more marked one of group B among tabetic and paretic patients may be due to the fact that syphilitic patients of groups AB and B have a positive Wassermann reaction, which in most of the cases persists in spite of all efforts to make it negative. Group B was present in 9.3 per cent of the insane persons with blue eyes and blond hair, while it was found in 20.8 per cent of those with gray eyes and brunette hair and in 26.8 per cent in those with brown eyes and brunette hair.

W. C. HUEPER.

SPECIFIC AND SUBSPECIFIC ANTIBODIES. W. MARKOFF, Ztschr. f. Immunitätsforsch. u. exper. Therap. **56**:95, 1928.

Antibodies of different character are produced if unchanged bacteria or bacteria more or less decomposed by bacteriophagic or fermentative action are introduced into the organism. Markoff differentiates between three types of antibodies. Antibodies of first degree are produced by the injection of unchanged bacteria. They possess a haptophoric group and are specific in action on the corresponding antigen. Complement-fixating substances, specific agglutinins, precipitins, etc., belong to this group. Antibodies of second and third degree are called subspecific antibodies. Antibodies of second degree resemble those substances effective in the protein therapy and follow the introduction of partly destroyed bacterial proteins. Partial agglutinins belong to this group. Antibodies of third degree result from the introduction of still more decomposed bacterial proteins. They react with group heterogenous proteins. The normal agglutinins and precipitins belong probably in this group. They are similar to the substances obtained in the nonspecific protoplasma activation after Weichardt.

W. C. HUEPER.

EXPERIMENTAL INVESTIGATIONS INTO THE GROUP SPECIFIC ANTIGENS AND ANTIBODIES. GREGOR GREENFIELD, Ztschr. f. Immunitätsforsch. u. exper. Therap. **56**:107, 1928.

Erythrocytes of group A gave a group specific complement-fixation test with some human serums. Alcoholic extracts gave even stronger reactions. The specificity could also be demonstrated by absorption tests. A group specific reaction was also obtained with saliva as antigen. Group specific precipitin reactions with alcoholic extracts could not be shown. The serum of rabbits injected with erythrocytes of group A contained species specific and group specific antibodies. Normal cattle serums treated with human erythrocytes of group AB agglutinated in twenty-three of fifty-four cases still erythrocytes of group O. The reaction was more marked at room temperature than at 2 C. Erythrocytes of group A and B showed occasionally also a weak agglutination. The anti-O-agglutinin could be separated again after its fixation on erythrocytes of groups O, A and B. Hearts of group O bound more strongly the anti-O-agglutin than hearts of group AB. Hearts of group A took an intermediary position.

W. C. HUEPER.

THE HETEROGENOUS SYSTEM IN HUMAN ERYTHROCYTES AND THE HETEROGENOUS HUMAN ANTIGEN IN ERYTHROCYTES OF ANIMALS. I. L. KRITSCHEWSKI and R. E. MESSIK, Ztschr. f. Immunitätsforsch. u. exper. Therap. **56**: 130, 1928.

The Forssman's antigen (sheep antigen) is present in erythrocytes of all men, independently from their blood groups. The human erythrocytes contain more-

over the antigens of Kritschewski (chicken), of Friede (turtle), of Friede and Gruenbaum (cat) and that of Landsteiner-van der Scheer-Witebsky (hog). The heterogenous human antigen and that of hog is present in erythrocytes of sheep, chicken, turtle and cat.

W. C. HUEPER.

THE SIGNIFICANCE OF LIPOIDS IN IMMUNIZATION. Ztschr. f. Immunitätsforsch. u. exper. Therap. **56**:191, 1928.

The lipoids of normal serum, as well as those extracted from the liver and purified possess identical qualities and act in the same manner on bacterial and snake poisons and bacteria by binding and detoxicating the former, and by decreasing the toxicity of the latter. The production of lipoids by the liver demonstrates the importance of this organ in the defense of the organism against intoxications and infections. The lipoids act on the toxins in twofold manner. They fix these substances and detoxicate them, producing atoxic products with immunizing properties. The modifying effect of the lipoids on the toxins is considered by the authors as an important factor for the production of vaccination substances of toxic character as that is demonstrated by the modification of the toxicity of anthrax bacilli.

W. C. HUEPER.

Tumors

THE CATALASE OF MALIGNANT TISSUE. M. R. LEWIS and H. COSSMAN, Am. J. Physiol. **87**:584, 1929.

It was found that extracts of inoculable chicken sarcoma of the Rous type were weak in catalase, in this respect resembling the muscle which the tumor invades. They also depressed, although they did not prevent, catalytic activity of extracts of other organs. Inactivation of the virus was affected by small amounts of peroxide, but the effect of this on the living tumor could not be determined, owing to the fact that the peroxide was promptly absorbed by the blood stream, with the production of fatal oxygen emboli. Rat sarcoma and carcinoma were more active in respect to catalase than was the chicken tumor.

H. E. EGGERS.

THE RELATION OF HEREDITY TO CANCER. MAUD SLYE, J. Cancer Research **12**:83, 1928.

The article is a polemic discussion with C. C. Little. It brings out in great detail the ideas of Slye concerning the relationship between carcinoma and heredity.

B. M. FRIED.

MALIGNANT LYMPHOBLASTOMA. J. S. MCCARTNEY, JR., J. Cancer Research **12**:195, 1928.

A case of malignant lymphoblastoma is described in which the organs showed a variable histologic structure corresponding in different areas to Hodgkins' disease, aleukemia, lymphoblastoma and endothelioma. Another case of a pure endothelioma of the lymph nodes is given in detail.

B. M. FRIED.

THE NONGENETIC APPEARANCE OF VARIOUS TYPES OF NEOPLASIA IN EXPERIMENTAL ANIMALS? L. C. STRONG, J. Cancer Research **12**:208, 1928.

Strong affirms that the teachings that all types of neoplasia occurring in laboratory mice are brought about by the activity or functioning of a single mendelian determinant are not convincing. The data of Maud Slye are believed by him to be neither consistent within themselves, nor with the more convincing data that have been accumulated by geneticists throughout the world in the last years. He believes that the process of heredity is merely a means to an end and by its investigation one is able to throw some light on the neoplastic changes

that lead to the occurrence of neoplasia, then one may still believe that the science of genetics may throw some light on this phenomenon. His argument is substantiated by a report of a study of three types of tumors derived from the connective tissue element of the embryo.

B. M. FRIED.

THE INFLUENCE OF THE ALIMENTARY REGIMEN ON TAR CANCER. J. MAISIN and A. FRANSOIS, Ann. de méd. **24**:455, 1928.

The authors affirm that diet has an influence on the development and the growth of a tar carcinoma. A liver diet accelerates the rate of growth of a tumor making it malignant. The active substance present in the liver resists desiccation and remains potent in a dry state for several months. The nature of this substance is unknown to them.

B. M. FRIED.

MULTIPLE CARCINOMAS. M. GOLDZIEHER, Virchows Arch. f. path. Anat. **267**:326, 1928.

A case is described in which three independent carcinomas were present: a squamous cell carcinoma of the esophagus with metastases of the lymph node, a typical liver cell carcinoma with rupture into the portal vein, and a tubular adenocarcinoma in the liver, of cholangiocellular type. The origin of the last mentioned tumor is discussed, and is attributed to proliferated liver cells which have given rise to pseudobile ducts.

B. R. LOVETT.

GENERALIZED LYMPHATIC VESSEL CARCINOMATOSIS WITH CHYLOUS ASCITES. K. SCHMÜCKER, Virchows Arch. f. path. Anat. **267**:339, 1928.

The author describes a carcinoma of the stomach, with extension into the lymphatics of the mesentery and abdominal organs, and farther into those of the entire body, especially of the skin. This extension could be explained by proliferation of the tumor cells in the lymph channels, with little tendency to tissue destruction. There was an extensive chylous ascites, due to diapedesis of chyle through the uninjured vessel walls. The milky character of the pleural exudate and the skin edema was caused by passage through the vessel walls of emulsified products of degeneration, arising from fatty changes and necrosis in the carcinoma cells.

B. R. LOVETT.

A CARCINOMA IN A THREE YEAR OLD CHILD. P. ROSTOCK, Virchows Arch. f. path. Anat. **267**:352, 1928.

A tumor of the sacrum, removed from a child, aged 3, proved to be an adenocarcinoma. This was not a carcinoma of the rectum in the ordinary sense, but may have originated from remains of the embryonic caudal gut.

B. R. LOVETT.

AN UNUSUAL TUMOR OF THE PANCREAS. A. PRIESEL, Virchows Arch. f. path. Anat. **267**:354, 1928.

An egg-sized, encapsulated tumor was found in the caudal end of the pancreas in a woman, aged 29. This was a solid epithelial growth, composed of large, finely granulated cells, and of at least limited malignancy, shown by the tendency of the cells to grow into the capsule and the presence of many mitotic figures. The origin of the growth was doubtful, probably either from the pancreas itself, or from misplaced rests of suprarenal cortex or liver. The cells bore the greatest resemblance to those of the liver.

B. R. LOVETT.

MALIGNANT SACROCOCCYGEAL CHORDOMA. J. PODLAHA and F. PAVLICA, Virchows Arch. f. path. Anat. **267**:363, 1928.

Including the tumor here described, forty-four instances of sacrococcygeal chordoma have been reported in the literature. The tumor consists typically of

a mass of fibrous stroma surrounding the parenchyma, in which the characteristic cells and a basophilic exoplasm are found. The cells lie singly, in masses or in alveoli, with intercellular substance between the groups, resembling a carcinoma more than a sarcoma. Vesicular cells are found, containing either a single large vesicle or many small vacuoles. A second type of cell is the small angular cell, with eosinophilic protoplasm. Cysts are also seen within the tumor, surrounded by the mucous-like exoplasm, and apparently arising from destruction of the cells. The mass is rich in glycogen and fat. The malignacy of this tumor consists in its tendency to recur after removal; the growth is slow and metastases rare.

B. R. Lovett.

Medicolegal Pathology

Embolism and Thrombosis of the Abdominal Aorta. M. M. Banowitch and G. H. Ira, M. Clin. N. Amer. **11**:973, 1928.

In five patients, all women, the symptoms were very similar: an acute onset with shock and severe pain, tenderness, loss of sensation and paralysis of the lower extremities which subsequently became cyanotic and gangrenous. One woman had a decompensated heart resulting from arteriosclerosis and hypertension. In the other four there was mitral stenosis. Postmortem examinations of the bodies of two of the four were the only ones secured. The clots found at the bifurcation of the aorta were regarded as emboli from the heart with subsequent thrombosis into the femoral and other arteries.

Because the symptoms were so much alike the authors believe that riding emboli from the heart lodged at the distal end of the aorta in all of the five women and occluded one or both common iliac arteries. In three, embolism of other vessels preceded that of the aorta. A most important symptom is loss of pulsation in the arteries of the lower extremities. About 105 cases of obstruction of the aorta by thrombi or emboli, chiefly the latter, have been reported and during recent years the clots have been surgically removed a few times with complete recovery.

E. R. Le Count.

Toxic Hepatitis and Hepatolysis Following the Use of Atophan. M. A. Rabinowitz, M. Clin. N. Amer. **11**:1025, 1928.

Three more cases of poisoning from cinchophen, all followed by recovery, are added to the three deaths and nine recoveries already reported, making fifteen cases altogether. Apparently the toxicity of the drug is due to the benzene rings it possesses. The symptoms are those of acute yellow atrophy. The liver of a woman who had taken 7½ grains (0.49 Gm.) three times a day for five months was greatly shrunken and practically without normal liver tissue. Rabinowitz also mentions five poisonings with two deaths from an iodine derivative of cinchophen (diiodatophan) which has been used for cholecystography. Cinchophen is especially dangerous when given to alcoholic persons, pregnant women, persons suffering from malnutrition or from other diseases associated with damage of, or a lessened amount of glycogen in, the liver.

E. R. Le Count.

Ergot Poisoning Among Rye Bread Users. J. Robertson and H. T. Ashby, Brit. M. J. **1**:302, 1928.

Gangrene Following the Use of Ergotized Rye Bread. W. J. Dilling and R. E. Kelly, Brit. M. J. **1**:540, 1928.

Among the Polish and German Jews in Manchester many poisonings from ergot were observed. They were found exclusively among those who ate bread made from rye harvested in the autumn of 1927, and grown during a cold, damp season. The ergot was found, identified by its physiologic reactions and the fungus was cultivated from the grain. Formication was an important symptom. One man

had gangrene of both hands. These observations from Manchester evidently stimulated the publication of the report by Dilling and Kelly which they made in Liverpool in 1923. This concerned symmetrical gangrene of the second toes requiring amputation of one in November, 1921, and of the other a year later. Ergot is most potent in flour made soon after harvesting and loses its strength as the grain drys. The patient was a Polish Jew, a salesman, aged 35; the ergot was found in the rye he ate. These writers emphasize the need of care in excluding Raynaud's disease and the obliterating thrombo-angiitis which occurs almost exclusively in Jews.

E. R. Le Count.

Acute Suppurative Leptomeningitis Due to Trauma Without Demonstrable Wounds. E. Ehrnrooth, Deutsche Ztschr. f. d. ges. gerichtl. Med. **12**:30, 1928.

There is but little adverse opinion to the important rôle trauma may play in causing the development of active syphilis or tuberculosis of the brain or of some tumors of the brain. With regard to the first two, it is believed that infection of the site injured occurs due to organisms in the blood stream. The injury of the head by direct or transmitted violence may be unaccompanied by any external wound, or the wound present may be insignificant. But there is little in the medical literature regarding a causal relationship between external violence which leaves no trace and infection of the brain or leptomeninges with ordinary pyogenic bacteria. A few cases have been reported in which it is claimed the bacteria gained entrance to the cranial cavity through existing fissures or crevices, or by lymph channels, and had their passage aided by trauma which left no wound or other injury. It is highly essential that symptoms should appear promptly after the injury to assume that violence has any part in causing the disease.

Ehrnrooth reports a meningitis which developed at once after a fall. The soldier, aged 20, struck his head when he fell; he was unconscious and headaches began that night. Death occurred two days later, and the meningitis was pneumococcal. The route of infection, presumably from some of the accessory nasal sinuses, was not ascertained at the postmortem examination. By putting virulent streptococci, staphylococci or pneumococci into the saphenous or ear veins of rabbits at the time when the animals were also hit on the head, the author found infection of the brain or its meninges in 68 of 152 animals used. He is not disposed, however, to apply these results in rabbits rigorously to similar occurrences in human beings.

E. R. Le Count.

Murder by Removal of the Bowel. L. Hirsch and M. Kresiment, Deutsche Ztschr. f. d. ges. gerichtl. Med. **12**:87, 1928.

Almost the entire bowel together with the sac of a large umbilical hernia mistaken for a tumor have been amputated from a newly born infant. Large portions of the small bowel prolapsing through wounds of the uterus or vaginal fornix have been removed by abortionists. Insane persons (Loessel and Jaki: *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **8**:419, 1926) have stripped the lining of the colon away from the rest of the bowel, pulling it out through the rectum; this has also been done by persons not insane (Schackwitz: *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **10**:31, 1927). Apparently removal of the colon lining in this manner is easily accomplished and causes little pain or none at all.

Hirsch and Kresiment report the murder of a girl, aged 11, by her insane mother. The child was first hit on the head and then, with her hands alone, the mother tore out the perineum, the adjacent walls of the vagina and rectum, the uterus and its adnexa, the distal half of the colon and all of the small bowel except the duodenum. The child was illegitimate. The report is devoted chiefly to the insanity of the mother.

E. R. Le Count.

Technical

BRILLIANT-GREEN ENRICHMENT METHODS IN THE EXAMINATION OF FECES FOR ORGANISMS OF THE ENTERIC GROUP. RUTH GILBERT and MARION COLEMAN, J. Infect. Dis. **44**:21, 1929.

This study would indicate that, for specimens which may be in transit twenty-four hours or more during submission to a laboratory, presentation in 30 per cent glycerol solution followed on recipt by inoculation of six plates of differential mediums would be a slightly better procedure for the isolation of *B. typhosus* than the Havens method. The preparation of the brilliant-green bile solution requires careful standardization, and irregularities may follow its use if its preparation is not adequately supervised. The use of either the Rakieten and Rettger or the Havens enrichment method in addition to the routine procedure of the New York State Laboratories would furnish a slightly higher percentage of successful isolations. Regardless of the method used, the submission of a series of specimens from each individual would seem to provide the best opportunity for the detection of typhoid carriers.

AUTHORS' SUMMARY.

THE PRESERVATION OF BLOOD FOR SUGAR ESTIMATIONS. H. LAX and I. SZIRMAT, München. med. Wchnschr. **76**:58, 1929.

The glycolysis and bacterial destruction of the sugar in diabetic and normal blood are prevented by the addition of 1 per cent sodium fluoride and 0.1 per cent mercuric chloride. With the addition of these reagents the sugar content of the blood remained unchanged even after thirty days' storage in the incubator.

AUTHORS' SUMMARY.

SIMPLIFIED METHOD FOR BACTERIAL INDOL. N. A. KOVÁCS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **55**:311, 1928.

P-dimethylamidobenzaldehyde (Merck), 5 Gm., is dissolved in pure amyl alcohol, 75 cc., and concentrated hydrochloric acid, 25 cc.; from 25 to 30 drops of this solution are added to broth cultures of bacteria. After a gentle shaking a violet-red color appears if indol is present in the broth.

W. C. HUEPER.

STAINING SPIROCHAETA PALLIDA IN FROZEN SECTIONS OF CENTRAL NERVOUS SYSTEM. R. KANZLER, Ztschr. f. d. ges. Neurol. u. Psychiat. **117**:171, 1928.

Kanzler has modified the method of Jahnel as follows: The section is immersed for thirty minutes in a solution of ammonium bromide and formaldehyde. It is then washed and put in pyridine for fifteen minutes, washed again and immersed for ten minutes in a 0.5 per cent uranin solution. After being washed again, the sections are immersed for one hour in a 1.5 per cent silver nitrate solution at from 37 to 40 C., quickly heated in the silver solution over the flame, washed in distilled water, dipped and moved about for from two to four seconds in a solution of silver nitrate, soda and ammonia. Then, without being washed, they are placed for from three to five seconds in a 5 per cent formaldehyde solution. As soon as they have taken on a yellow or yellowish-brown color, they are placed in distilled water, which is changed several times, and consecutively in alcohol, beechwood creosote-phenol-xylene, and Canada balsam. The spirochetes are stained black, whereas the nervous tissue remains unstained.

Society Transactions

NEW YORK PATHOLOGICAL SOCIETY

Anniversary Meeting, Jan. 10, 1929

HARRISON MARTLAND, M.D., *in the Chair*

ENDOCARDITIS IN MALTA FEVER (UNDULANT FEVER). C. E. DE LA CHAPELLE.

An Italian laborer, aged 38, was admitted to Bellevue Hospital, third medical division, complaining of fever. His past history revealed the important information that, while in Italy nine months previous to admission to the hospital, he drank goat's milk. He arrived in the United States one month later and was admitted to a hospital where a diagnosis of typhoid fever was made. After five months in the same hospital, he left and went to the country where he felt well for about three weeks. Attacks of chills, fever and headache, followed by pains throughout the body, began about that time. Several weeks before admission to Bellevue Hospital the small toe of the right foot became extremely painful and began to turn black.

On physical examination he presented dyspnea and a café au lait color of the skin. The small toe of the right foot was red, with some black discoloration; it was swollen and was extremely tender to touch. Petechiae were also present on the fourth toe. Both feet were edematous. The fingers presented definite clubbing. The heart was slightly enlarged; the first sound at the apex was of poor quality. A distant, musical systolic murmur was heard at the apex and transmitted to the left; another soft systolic murmur was elicited at the aortic area. The rhythm was regular; the rate, 96. The temperature was 101 F. The systolic blood pressure was 90; the diastolic, 60. Both the spleen and the liver were palpable and tender.

Several days later an Osler's node was observed on the tip of the fourth finger of the right hand. Three weeks later the patient complained of pain in the right thigh. The femoral pulse was scarcely palpable in this leg. Shortly before death some few days later, he complained of severe pain in the lower part of the abdomen and both groins.

The clinical diagnosis was: (1) heart disease, *(a)* bacterial infection, active; *(b)* endocarditis, subacute; *(c)* regular sinus rhythm; *(d)* class 3; and (2) Malta fever.

Urinalysis revealed a trace of albumin, hyaline and granular casts, and an occasional white blood cell but no red blood cells. On admission, the patient had 3,930,000 red cells, with 48 per cent hemoglobin and 3,350 white blood cells with 69 per cent polymorphonuclears and 26 per cent lymphocytes. Just before death, the red cells numbered 2,600,000 with 60 per cent hemoglobin, and the white cells 2,200. The Wassermann reaction was negative.

Blood cultures taken on two separate occasions revealed a small, gram-negative, nonmotile organism, so small that its outline could not be clearly defined. These cultures, together with serum taken during life, were studied by Dr. Charles Carpenter of the Diagnostic Laboratory of the New York State Veterinary College and reported by him as being a true type of *Brucella melitensis* A.

At necropsy the outstanding observations were as follows: massive vegetative and ulcerative endocarditis of the aortic valve; massive septic splenomegaly with multiple anemic infarcts; hemorrhagic glomerular and tubular nephritis; parenchymatous degeneration of the liver; massive subperitoneal hemorrhage in the right half of the abdomen, of unknown origin, and subungual petechial hemorrhages.

The heart appeared to be about normal in size and weighed 345 Gm. The two anterior aortic cusps were practically completely destroyed and replaced by a soli-

tary, creamish-white, granular mass of fused vegetations, irregularly round, rather soft and friable and approximating the size of a thumb. The posterior aortic cusp was distinctly thickened, and on its ventricular aspect, at about its center, presented a vegetation, the size of a pea, which was loosely attached and fell away apparently of its own weight. The muscle of the heart was pinkish and opaque, but was otherwise well preserved. The aorta was excellently preserved.

The patient undoubtedly became infected in Italy by drinking goat's milk shortly before coming to this country. The onset of the symptoms occurred about one month after his arrival here, at which time he was admitted to a hospital where a diagnosis of typhoid fever was made. The duration of the malady, therefore, was about nine months, simulating the subacute course of *Streptococcus viridans* endocarditis. The clubbing of the fingers, the enlarged spleen, the café au lait color of the skin and the embolic manifestations completed the picture of subacute bacterial endocarditis.

DISCUSSION

HARRISON S. MARTLAND: I suppose that without complete bacteriologic identification this case might easily pass as an influenzal endocarditis. I believe that in about 5 per cent of Dr. Libman's original series of subacute bacterial endocarditis the condition was attributed to *B. influenzae*.

WARD J. MACNEAL: I should like to call attention to the rather difficult bacteriology of infections of this type. The prevalence of contagious abortion in dairy cattle is not well recognized and not sufficiently well known to the various workers in animal husbandry. Previous to 1909, I believe that all authorities on dairy husbandry doubted the existence of contagious abortion in cattle in the United States. In 1910, one of my assistants and myself isolated *Brucella abortus* of Bang from an aborting cow at the Illinois Agricultural Experiment Station. (MacNeal, W. J., and Kerr, J. E.: Bacillus abortus of Bang, the Cause of Contagious Abortion in Cattle, J. Infect. Dis. **7**: 469, 1910.) This observation was reported at the subsequent meeting of the Society of American Bacteriologists at Ithaca, New York. The bacteriologists and veterinary pathologists present at this meeting were wholly unfamiliar with this organism at that time. Since then it has been shown that this condition is widespread in dairy cattle in this country. Apparently, the variety of microbe which is present in cattle is somewhat less virulent for man than is the variety which is present in goats. The bacteriologic distinction is not easy, but there seems to be a difference in virulence. The recognition of human infection due to the cattle type is rather rare. I presume there are less than 100 cases in the literature. It would seem that we have here a good reason for the pasteurization of milk in general, especially because of the difficulty of recognizing the disease when it exists in the cattle. Sometimes in pure-bred herds of cattle, producing the highest grade of milk, contagious abortion will be unexpectedly discovered. It seems to me that we should be cautious about the possibility of this disease existing in cattle, especially in dairy herds; it is difficult to recognize.

THE SIZE OF THE CONSOLIDATED LUNG IN LOBAR PNEUMONIA. P. N. CORYLLOS and GEORGE L. BIRNBAUM.

Are the consolidated lobes in lobar pneumonia larger than the healthy ones? Affirmative answers have been given by Barth (Dictionnaire des sciences médicales, 1888, vol. 26, p. 227), Blake (quoted by Cecil: Text Book of Medicine), Leslie (Leslie and Alexander: Pneumonia, 1924, p. 47) and Aschoff (Pathologic Anatomy, 1923, vol. 2, p. 284) and in textbooks on medicine and pathology. MacCallum (Text Book of Pathology, 1928, p. 517) and Delafield and Prudden (Text Book of Pathology, 1928) are noncommittal on this phase of the subject.

We are of the opinion that the affected lobes are smaller than the sound ones, basing this contention on experimental and clinical evidence. (Coryllos and Birnbaum: Arch. Surg. **16**:501 [Feb.] 1928; ibid. **18**:190 [Jan.] 1928; Bull. N. Y. Acad. Med. **4**:383, 1928). In making autopsies on pneumonic dogs we first clamped the trachea before opening the chest and found this to be so. The pneumonic lung was

smaller and bluish black and was sunken toward the posterior part of the chest cavity. The size of the healthy lung was not here due to an expansion of the thorax, for these animals died with the chest in a phase of extreme expiration. The state of expansion of the sound lung was therefore dependent on and proportional to the shrinkage of the pneumonic lung. If the tracheal clamp was then released, the healthy lung collapsed to a smaller size than the diseased one. (This experiment was repeated at the meeting of the New York Pathological Society, Academy of Medicine, Jan. 10, 1929.) These observations were clinically verified at autopsy of a man, aged 60, who died on the sixth day of the disease with pneumonia of the entire right lung. With the trachea clamped the chest was opened, and the left (healthy) lung appeared larger than the right; this impression was confirmed by measuring the water displacements of the respective lungs. It is necessary to proceed rapidly and avoid manipulation of the healthy lung because air diffuses rapidly through its alveolar surface. Clamping the trachea maintains the true relative size of the respective lungs uninfluenced by the opening of the chest with the consequent disappearance of the intrapleural negative pressure.

That such observations are to be found at autopsy is not surprising when it is considered that in the dog experimentally produced lobar atelectasis and lobar pneumonia are indistinguishable from one another by roentgenogram or even by the gross appearance of the lungs. Moreover, injection studies (to be published) of the pulmonary circulation show that there is an initial shrinkage of the alveoli in both diseases and later a marked impairment of the capillary circulation.

Against the view that the pneumonic lung is larger than the affected one is the fact that the heart and mediastinum have never been reported shifted toward the sound side in an uncomplicated case. On the contrary, shifting of the heart and trachea toward the affected side with elevation of the homolateral diaphragm (cardinal sign of atelectasis) has been reported in cases of lobar pneumonia in children by Thoenes (Monatschr. f. Kinderh. **22**:353, 1924), Wallgren (Acta pediat. **3**:81, 1922), St. Engel (Handb. d. Kinderh. [Pfaundler and Schlossmann] **3**:636, 1924) and Griffith (Am. J. M. Sc. **174**:448, 1927). However, the dictum of absolute rest and immobility for the patient, so generally ascribed to, has left the medical profession in sore need of such further useful data as could come from early serial roentgenograms in adults. Moreover, one seldom obtains postmortem examinations at the beginning of the disease, and knowledge about the early pathologic process is therefore limited.

We have thus come to regard lobar pneumonia as essentially a pneumococcic lobar atelectasis. That is, whereas lobar atelectasis is considered dependent on occlusion of a lobar bronchus (and absorption of alveolar air) with a relatively sterile and nontoxic bronchial exudate, lobar pneumonia is considered as a lobar atelectasis occasioned by occlusion of lobar bronchus with a viscid fibronous exudate laden with pneumococci the toxins of which poison the patient. In the latter instance it should be remembered that the concomitant alveolar exudation in pneumonia prevents as great a shrinkage of the lung as occurs in simple lobar atelectasis, spontaneous or after operation. At autopsy, therefore, the relative difference in size of the pneumonic and the healthy lung need not be so striking, especially in a late stage of the disease when there is already considerable alveolar exudate.

DISCUSSION

GEORGE L. BIRNBAUM: I wonder whether it has occurred to many what the cause of the indentations of the ribs in the pneumonic lung may be other than enlargement. There is not much question that the indentations are there. It seems to me that they can be accounted for by the simple explanation of gravity. In this way these indentations would mean pressure and weight of the edematous lung against the thoracic cage, rather than enlargement of the affected lobe.

HARRISON S. MARTLAND: The paper presented is important. It is indeed gratifying to encounter research in pathology along purely mechanical, physical or chemicophysical lines and free from weird immunologic theories. Dr. Coryllos and Dr. Birnbaum have undoubtedly proved that the pneumonic lung is smaller

than the uninvolved lung. For some time I have even thought that resolution in lobar pneumonia was due almost entirely to mechanical factors, and had little to do with special immunologic phenomenon.

CHICAGO PATHOLOGICAL SOCIETY

Regular Monthly Meeting, Jan. 14, 1929

ESMOND R. LONG, *President, in the Chair*

EXPERIMENTAL STUDIES ON COLLAPSE OF THE LUNGS IN THE RABBIT. ROBERT G. BLOCH.

These experiments were undertaken to study: first, the mechanical effects of pneumothorax on the chest of the rabbit; second, the influence of the pleura, and third, the respiratory changes. Collapse of the lung was caused in seventy normal rabbits over periods varying from five minutes to eleven months.

The mediastinal structure does not permit leakage of air from the air-filled pleural cavity into the opposite side, but the mediastinum is extremely labile, and complete collapse of the lung displaces it to an extent which is not observed in man. The heart does not rest on the diaphragm, but is suspended in the center of the thorax with two mediastinal pillars connecting it with the diaphragm. The pillars are under slight tension and are easily movable. They include a cavity which is filled by an additional (fourth) lobe of the right lung. The diaphragm is flabby and gives way to intrapleural pressure more easily than in man. It is concluded that the anatomic conditions in the chests of rabbits and of human beings are too different to apply the results clinically.

Pneumothorax was maintained in most of the rabbits with intrapleural pressure of about +1 cm. of water which corresponds to a collapse under fairly high positive pressure in man. Atmospheric air was used for collapse. It is absorbed rapidly. The average rabbit's lung reexpands completely within twenty-four hours after complete collapse. Refillings had to be done daily at first to maintain pneumothorax. The intervals between fillings could be lengthened after about one month and were gradually increased to seven days, which was found to be the maximum time that the lung will stay collapsed. A marked noninflammatory thickening of the visceral pleura occurs, increasing rapidly after collapse of one month's duration. No pleural exudation was observed. No formation of adhesions occurred after the lung was permitted to reexpand. In every case, it was easy to recollapse. The absorption of air is directly proportional to the thickness of the pleura, and vice versa, the thickening of the pleura is most marked where it is most exposed to contact with the inflated air.

The experiments on the respiratory changes due to collapse of the lung were instigated by the recent practice of simultaneous bilateral pneumothorax. The abdominal and thoracic respiration were recorded during the procedure of collapse and afterward up to twenty-two hours, simultaneously with the intrapleural pressure. Even extreme collapse does not change the abdominal respiration materially, but increases the thoracic respiration to five or six times its normal volume. The change in respiration is not a shock effect, but the actual response to air hunger. It follows readily the repeated removal and reinflation of air. The results suggest that careful study should be made of the respiratory response of patients when collapse of the lung occurs before simultaneous bilateral pneumothorax is clinically accepted.

DISCUSSION

E. R. LONG: Is the opening of the alveoli under the pleura due to absorption of air from the pleural space, or was the periphery of the lung incompletely collapsed?

R. G. BLOCH: The peripheral portions of the lung were collapsed.

A Microscopic Carcinoma of the Testis Concealed in Chronic Granulation Tissue. Paul J. Breslich.

This tumor in the testis of a man, aged 24, was concealed in a mass of dense fibrous tissue in which, with the tumor cells, were regions of chronic granulation tissue closely resembling that stimulated by *B. tuberculosis*. In the center of certain of these masses were necrotic cells or cellular detritus and ingrown epithelium arranged in bands and cords like liver trabeculi with little stroma. The stimulus for this unusual growth of granulation tissue may have been the chronic irritation produced by substances derived from testis tissue destroyed by the invading tumor, or liberated by necrosis of the carcinoma cells. Substances likely to cause this reaction are the lipins, especially cholesterol. Acid-fast bacilli were not found in these tissues.

Experimental Study of Some Immune Reactions to Ascaris. Hamilton R. Fishback.

Various extracts of dried ascaris powder were used directly and after incubation with immune rabbit serum. The hemolytic action of saline and iodized oil 40 per cent extracts against human red blood cells was completely neutralized by the immune serum. The stimulating action on a uterine strip from a sensitized guinea-pig and the reactive effect on the skin of a sensitive human subject of the saline extract of *Ascaris* were not lessened by the immune serum, while the same activities of the iodized oil extract were inhibited by the immune serum.

DISCUSSION

B. L. Rappaport: The relation of human ascaris to hog ascaris is an interesting problem. There is no morphologic difference. Serologic tests have failed to differentiate.

Studies in Experimental Calcification. Aaron Learner.

M. B. Schmidt, in his discussion of pathologic calcifications, divides them into two main groups:

(1) Califications occurring where there have been local tissue disturbances without any change in the calcium metabolism; (2) calcifications occurring in healthy tissues as a result of changes in the calcium metabolism.

The important conditions in the process are: *(a)* oversaturation of blood and tissue fluid with calcium; *(b)* inability on the part of the excretory organs to excrete calcium satisfactorily, and *(c)* changes in solubility relations in the blood for calcium.

From the literature the following statements illustrate one or the other or several of these conditions.

Hofmeister stated that a decrease in carbon dioxide will lead to the precipitation of calcium when calcium supersaturation exists because of carbon dioxide increase. Such fluctuations by alternately varying the reaction, at the intervals of two days, with diets the ash of which was respectively acid and alkaline, led to metastatic calcification in experiments by Rable and Dreyfuss. Calcium was deposited in practically all of the tissues of the white mice.

Katase, on the other hand, succeeded in obtaining metastatic calcification in guinea-pigs and rabbits by simple injection of various calcium salts. Calcium was deposited in all the tissues, but of especial interest is his description of calcium in the bronchial mucosa, and in the elastica, and he suggested that calcium is excreted in part through the bronchial mucosa. The two conditions that he considered important are oversaturation with calcium and also interference to some extent with the excretion of calcium.

Parathyroid extract-Collip is an agent capable of mobilizing calcium. Given in large dosage it produces hypercalcemia, 100 per cent or more, with attendant symptoms such as anorexia, emesis, somnolence, circulatory weakness and death.

Two questions arise: What is the source of the calcium, and what are the pathologic consequences?

Stewart and Percival concluded, as had Greenwald and Gross, that the calcium enters the blood from the soft tissues or the bones, or both. The bones, however, are the most likely source of the calcium, and they may well be considered as a reservoir for calcium much as the subcutaneous tissues are considered reservoirs for fat which is released when needed.

To answer the question as to the pathologic consequences of an overdosage of parathyroid extract-Collip, the following experiments are presented.

Several dogs were given injections of from 100 to 150 units of this drug. The calcium value rose to 19.57 mg. per hundred cubic centimeters of blood. The dogs died with the symptoms mentioned. Microscopically, there was only a marked decrease in the coagulation time of the blood, a hypertonic state and hyperemia of the gastro-intestinal tract. Microscopically, calcium as demonstrated by Kossa's silver nitrate reaction, was found practically in all the organs and tissues of the body.

Especially interesting were those livers in which calcium was found in the liver cells and also in the Kupffer cells. In the lung, the calcium was in the elastica tissue of the alveoli and in the tunica propria of the bronchi. The epithelium was desquamated because of the large amount of calcium. In the kidney the calcium was present chiefly in the lumina of the tubules, in Bowman's space, and less so in the vessels and connective tissue.

From the standpoint of the conditions involved, the following facts apply: In the lung, stomach and heart, there was increased alkalinity consequent to the acids formed; and in the liver there was supersaturation, a factor also effective in all tissues. Inability of the excretory organs to cope satisfactorily with the excretion of calcium was another factor.

Of interest is the effect of the amount of calcium in the diet in the deposition of calcium in the tissues with and without associated injections of parathyroid extract-Collip.

Fifty mice were divided into three groups: (1) those fed a diet without calcium, (2) those on a diet with an amount of calcium equivalent to the salts of milk and (3) those on a diet containing twice the amount of calcium given in group two. Each group was divided into two parts, one part receiving injections, the other not. Each mouse received 17 units in five weeks; then a number of mice at one dose received 10 units. These are enormous doses. Süssmann, in determining the calcium variation in white mice by the use of parathyroid extract-Collip stated that 10 units per mouse of 20 Gm. is equivalent to giving to a man weighing 64 Kg. a dose of 20,000 units. He noted no clinical disturbances nor any rise in blood calcium. These chemical determinations are in accord with our histologic observations. Microscopically, no calcium was found in any of the organs except in the bronchial cartilages. But caution is necessary in interpretation, for calcified bronchial cartilages are found in normal mice though possibly not with the frequency observed in the experiments.

Interesting to note is the reported unresponsiveness of white mice and white rats to other hormonal substances, as reported by Voegtlin and Dyer in some experiments with epinephrine and pituitary extract.

Conclusions.—Mice are unresponsive to parathyroid extract-Collip.

In dogs, large doses of this drug lead to death and to metastatic calicification of the gastro-intestinal tract, heart, lungs, liver, kidneys, pancreas, etc.

The calcium in the lung and the arrangement in and under the bronchial mucosa gives morphologic support to Katase's suggestion that calcium is excreted in part through the bronchi.

Furthermore, the presence of calcium in the glomerular tufts and Bowman's space and in the lumina of the tubules gives definite morphologic support regarding the excretion of calcium in the kidney, namely, through the glomerular tufts.

DISCUSSION

DÉLA HALPERT: Was the calcium content of the bile determined?

AARON LEARNER: No.

R. H. JAFFÉ: The studies are important in demonstrating that calcium is eliminated through the bronchi and in the glomerular tufts of the kidneys.

Regular Meeting, Feb. 11, 1929

ESMOND R. LONG, *President*, in the Chair

DEATH OF DR. A. A. MAXIMOW. R. H. JAFFE.

Alexander Alexandrovitsch Maximow was born in St. Petersburg, Russia, on Jan. 22, 1874, the son of a well-to-do merchant. He attended the German high school there and entered the Imperial Military Academy of Medicine from which he graduated in 1899. After two years of postgraduate work in Germany, especially under Ernst Ziegler in Freiburg, he returned to his alma mater to engage in research work on the embryology of the blood and the histologic changes in inflamed connective tissue. His monograph on the inflammatory formation of connective tissue attracted much attention. At the age of 29 he became head of the department of histology and embryology at the university from which he had graduated. He held this position for nineteen years. In 1912, he received the degree of Doctor of Science from Trinity College, Ireland. The Russian revolution and the hectic years of the early Bolshevik regime interfered greatly with his work. As counselor to the Imperial Russian government and Physician General to the Imperial Russian army, he could not accustom himself to the development in his native country. When an opportunity was offered to come to the United States he gladly accepted. In the early spring of 1922, he escaped the spies surrounding him, made an adventurous flight over the Baltic Sea to Sweden from where he embarked for New York. In May, 1922, he was appointed professor of anatomy at the University of Chicago. It was in this position that he reached the peak of his productivity. In the midst of his work, engaged in numerous research problems he was called away. On the morning of Dec. 3, 1928, the unexpected, terrible message came that he had died suddenly. Postmortem examination revealed as the cause of his death a severe coronary sclerosis.

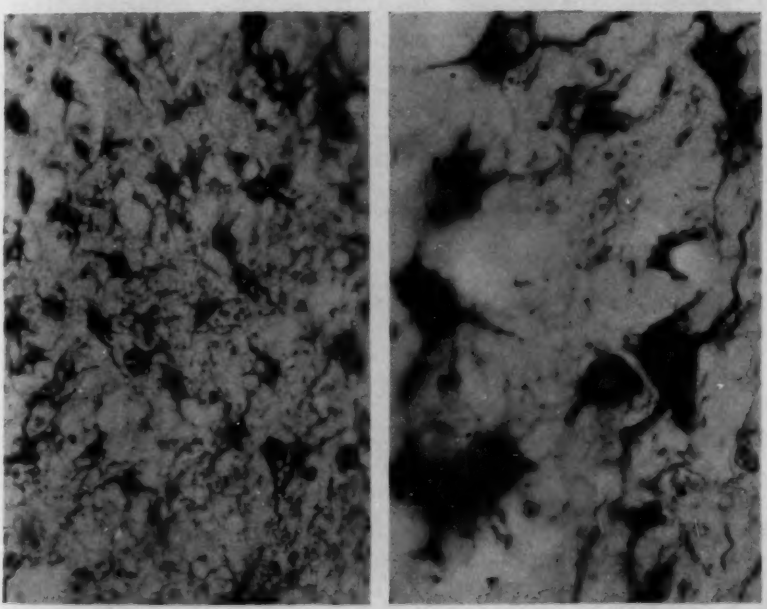
Maximow's life work was the study of the development of the blood cells, their relation to each other and to the cells of the connective tissue. Limited as the field appears at first glance, he made it a science in itself. Whenever new methods were discovered he applied them with rare technical skill in order to learn more about the resting wandering cells, lymphocytes and polyblasts. Vital staining and supravital staining and, during the last years especially, tissue cultures seemed to prove his conception which he had expressed already in his earliest publications and which he had fought for with great energy throughout his life; namely, that the small lymphocytes may become transformed into macrophages, monocytes and the other cells of the circulating blood. Time does not permit a list of all his contributions. Mention is made only of his recent studies: the formation of tubercles in tissue cultures, the behavior of the Calmette-Guerin tubercle bacillus in explanted tissue, the development of the reticular and collagenous fibers in tissue cultures, and the mesothelium.

In 1913-1914, Maximow published a textbook of histology in two volumes in Russian. The war prevented its translation and thus it did not become widely known. The lack of an adequate, modern and concise presentation of this subject in the English language induced Maximow to undertake the task of writing such a book. It was three-fourths completed at the time of his death. It is to be hoped, however, that the work will be completed as a monument to this great and genial man.

OLIGODENDROGLIOMAS OF THE BRAIN. PERCIVAL BAILEY and P. C. BUCY.

In 1924, in examining the gliomas in the collection of Dr. Harvey Cushing, one of us noted certain cellular tumors of slow growth whose nuclei resembled those of the oligodendroglia. The tumors contained a few neuroglial cells and much delicate intercellular material which could not be sharply stained or impregnated in any way. Although the tumors were believed to consist essentially of oligodendroglia and although they were called oligodendrogliomas (Bailey and Cushing: Tumors of the Glioma Group, 1926), positive proof could not be obtained because the pathologic specimens were not properly fixed to permit the use of Hortega's specific method.

Recently Globus (*Arch. Neurol. & Path.* **19**:263 [Aug.)] 1927) and Penfield (*Am. J. Path.* **4**:153, 1928) have so modified the method of Hortega that it can be used on material fixed in 10 per cent neutral formalin. Working with this



Oligodendroglioma. Globus-Penfield modification of the silver carbonate method of del Rio-Hortega. At the left, numerous fairly normal oligodendroglia; × 320. At the right, the same cells under a higher magnification; × 780.

modified method we first proved, in October, 1928, that a tumor of the brain was actually composed of oligoglia. Since then we have identified twelve other similar tumors.

The accompanying illustration shows typical cells from an oligodendroglioma of the type described. At the left are seen numerous cells resembling normal oligoglia; to the right the same cells appear under a higher magnification.

DISCUSSION

PETER BASSOE: How are these cells differentiated from neuroglia, and do they occur in all parts of the brain?

P. BAILEY: There are certain distinct differences in structure between the oligodendroglia and glia cells. The staining procedure was carefully controlled by normal tissues. These cells occur in all parts of the brain and spinal cord

wherever there are long medullated fibers. The tumors are rather avascular, tend to calcify, and are favorable for operation.

TRAUMATIC ANEURYSM OF THE AXILLARY ARTERY. E. L. MILOSLAVICH.

A man, aged 64, weighing about 230 pounds (104.3 Kg.), fell on his left side and dislocated the left humerus (subcoracoid luxation), which was reduced without particular difficulties. An excruciating pain which appeared at the time of the dislocation persisted for several days. About four weeks after the accident, a tumor the size of an orange appeared in the left armpit and grew gradually until it reached the size of a large grapefruit. This growth was clinically interpreted as a sarcoma. The patient died about three years after the accident with symptoms of pulmonary involvement which were believed to be sarcoma metastases. At no time was there any noticeable pulsation of the tumor nor were there any signs of circulatory disturbances in the left arm.

The autopsy demonstrated an aneurysm of the left axillary artery, 18 by 17 by 12 cm. Both axillary arteries showed an advanced atheromatosis with intimal ulcerations. Identical changes, but to a more pronounced degree, were present in the inner lining of the entire aorta. The heart showed an eccentric hypertrophy of its left ventricle but no valvular lesions.

At the time of the dislocation, an injury occurred to the already diseased (atheromatous) axillary artery, leading to a rupture of its inner layers (incomplete or inner rupture of the artery). A complete laceration of the artery did not occur at the time of the accident or during the surgical reduction as no hematoma was observed. The aneurysm developed gradually until it reached the aforementioned unusual size. Similar observations were made by Stimson, Koerte, Kraemer, Hoffmann and others. Aneurysm of the axillary artery is an uncommon but characteristic complication of the dislocation of the humerus.

DISCUSSION

R. H. JAFFE: It would seem that with so large an axillary aneurysm there must be some unusual disease of the vessel wall such as an arteritis. What did the microscopic examination demonstrate?

E. L. MILOSLAVICH: The only changes observed microscopically were those characteristic of senile atheromatous sclerosis.

PRIMARY CARCINOMA OF THE DUODENUM. H. D. COUNTRYMAN.

Carcinoma of the duodenum, according to Dewis and Morse (*New England J. Med.* **198**:383, 1928), was first reported by Hamberger in 1746. His patient had a scirrhus carcinoma of the first portion of the duodenum which perforated the wall and caused peritonitis. According to the report of Eusterman, Berkman and Swan (*Ann. Surg.* **82**:153, 1925), carcinoma of the duodenum constitutes about 1.5 per cent of all intestinal carcinomas which, in turn, compose 10 per cent of all carcinomas. Brill (*Am. J. M. Sc.* **128**:824, 1904) computed from the combined statistics of Maydle, Nothnagel, Mueller and Lubarsch that 0.3 per cent of all intestinal carcinomas occurred in the duodenum. Deaver and Radvin (*Am. J. M. Sc.* **159**:267, 1920) stated that carcinoma of the duodenum was responsible for 0.033 per cent of all deaths. Statistics vary as to the frequency of carcinoma in the anatomic divisions of the duodenum. This is probably because carcinomas of the second or intermediate portion are confused with those of the ampulla of Vater. According to Deaver and Radvin, 22.15 per cent occur in the pars superior, 65.82 per cent in the pars intermedius and 12.02 per cent in the pars inferior. Statistics taken from elsewhere do not entirely agree with these. Eusterman, Berkman and Swan reported six in the pars superior, six in the pars intermedius and three in the pars inferior. Dewis and Morse reported twelve carcinomas of the duodenum: five in the first portion, five in the second, and two in the third. Figures as to their relative frequency in sex are not conclusive, but it is generally held that there have been a few more in men than in women.

Most of the carcinomas occurred in persons between the ages of 50 and 60 and the youngest age recorded was a 16 year old patient of Ewald. Perry and Shaw (*Guy's Hosp. Rep.* **50**:214, 1893) observed one in a patient, aged 80. Etiologically, little more is known of duodenal carcinoma than of other forms. The greatest contention is whether or not duodenal carcinomas arise from duodenal ulcers. All of the views in regard to this subject are purely hypothetic, but it seems that duodenal ulcers have little etiologic importance. Duodenal ulcers occur frequently, whereas carcinoma is rare, and the greatest proportion of duodenal carcinomas occur in the second portion of the duodenum where ulcers are seldom found.

Grossly, carcinoma of the duodenum may be polypoid, infiltrative scirrhus which often is circular and narrows the lumen, or a soft, raised tissue. Histologically, they usually have a tubular structure. Next in frequency are those with an alveolar structure and an abundant fibrous stroma. Only one colloid carcinoma of the duodenum has been reported (Letulle: *Bull. Soc. anat. de Paris* **11**:721, 1897). The carcinoma which I report has a papillary structure.

Duodenal carcinoma may arise from the epithelium of the Lieberkühn crypts, Brunner's glands or pancreatic rests. Orator (*Arch. f. klin. Chir.* **134**:736, 1925) suggested that carcinomas of the pars superior may also arise from gastric mucosa which has extended into the duodenum. In a previous article he stated that gastric mucosa often extends for a short distance into the duodenum. He quoted Scagliosi who stated that many duodenal carcinomas result from a hyperplasia of Brunner's glands associated with ulcers.

Carcinoma of the duodenum as a rule form metastases slowly. In twelve reported by Dewis and Morse only three had spread widely, and two others had invaded the regional lymph gland. The secondary growths, when present, usually are found in the regional lymph glands, the liver and the lungs.

Report of Case.—M. H., aged 42, a salesman, entered the service of Dr. H. E. Jones, St. Luke's Hospital, on Aug. 13, 1926, because of weakness and jaundice for two months and attacks of dizziness for three days. He had measles at the age of 4 years and typhoid fever at 13. For a week he had had one or two tarry stools each day and had lost 50 pounds (22.7 Kg.). He was markedly jaundiced but well nourished, suffered no pain and weighed 225 pounds (102 Kg.). On the right side of the abdomen there was a palpable smooth mass which seemed to be connected with the liver but was not tender. Roentgen examination disclosed a dense shadow extending below the right leaf of the diaphragm. There were 1,945,000 erythrocytes, 17,250 leukocytes and 39 per cent hemoglobin. The coagulation time was four hours and twenty minutes. There was bile in the urine and occult blood in the stools. Following two blood transfusions a cholecystectomy was performed on Dec. 4, 1926. At the time of operation the gallbladder and common bile ducts were markedly dilated although a probe was passed readily into the duodenum. Abnormalities of the duodenum or stomach were not detected by palpation. The postoperative recovery was excellent. The patient gradually regained 20 pounds (9 Kg.) and his appetite returned. The jaundice which had been decreasing even before the operation continued to diminish. The patient was discharged and remained well until Dec. 10, 1927. When examined by Dr. Greer he was weak and anemic. The skin was not appreciably jaundiced, but bile was found in the urine, and blood in the stools. His condition progressively grew worse, and he lost considerable blood in the feces. He died on May 12, 1928, soon after receiving 500 cc. of blood and 500 cc. of normal physiologic solution of sodium chloride intravenously.

In the lining of the duodenum was an indurated cauliflower-like growth beginning 6 cm. below the pyloric ring on the posterior and left sides of the wall. The growth extended down the remainder of the pars intermedius and into the pars inferior for 3 cm., the entire length of the tumor being 12 cm. At its widest portion about on a level with the papilla of Vater it was 3.75 cm. wide; here it encircled one half of the circumference of the bowel. The margins were raised and wavy and the tissue in the center was friable and grayish pink. The papilla

of Vater was encroached on in the posterior edge of the growth. The common bile duct and pancreatic duct opened separately through small orifices into the lumen of the duodenum. At a level 2 cm. below the papilla the lumen of the duodenum was one-half obstructed by the tumor; elsewhere it was from one-fourth to one-third obstructed. On surfaces made by a transverse section through the duodenum and adjacent head of the pancreas, at a level 2 cm. below the papilla, the muscularis beneath the tumor was thin; in places the growth had penetrated and had infiltrated slightly the surrounding pancreatic tissue for a distance of 1 cm. The descending portion of the duodenum above the tumor was distended slightly and pulled up by fibrous adhesions attached to the gallbladder fossa. The biliary lymph glands formed an indurated mass 6 by 5 by 3 cm. The lymph glands along the superior edge of the pancreas to within 6 cm. of the tail were firm and as large as 1 cm. in diameter. Continuous with the enlarged biliary lymph glands were enlarged firm lymph glands around the right renal vessels. There were two or three small shotlike lymph glands along the greater curvature of the stomach. All the surfaces made by cutting these glands contained many tumor nodules. The tumor surrounded the terminal 1.75 cm. of the duodenal end of the common bile duct; proximal to this area the common bile duct was dilated so that at the level of the cystic duct it was 4.5 cm. in circumference. The hepatic ducts were markedly dilated. The lining of the common and hepatic bile ducts was everywhere smooth even where the common duct opened through the papilla of Vater. The lining of the pancreatic duct was also smooth although the lumen was greatly dilated. The growth completely encircled the walls of both the common duct and pancreatic duct in their course through the papilla of Vater, but the linings here were unaltered.

The receptaculum chylum contained in its dilated lumen a pearly-white papillary mass of tissue 1 by 1 by 1 cm. attached by a thin pedicle and resembling the egg masses of fish. There were also two constrictions in the thoracic duct completely encircling the wall and causing a puckering of the surrounding fat tissue. Each constriction was about 1 cm. long; one was in the midthoracic portion, the other just above the receptaculum chylum.

The lower margin of the liver was at the costal edge on the right side and it weighed 2,120 Gm. Both its external surface and surfaces made by cutting were studded with many white, irregular nodules as large as 1 cm. in diameter. The bile ducts were dilated. All the lobes of the lungs contained scattered tumor masses 1 to 2 mm. in diameter. The wall of the left ventricle of the heart was hypertrophied. There was an acute hyperplasia of the spleen, and the small and large bowel contained tarry feces.

Histologically, in sections of the duodenum taken from the edge of the tumor there was a small piece of mucosa with normal villi and Lieberkühn glands but with a stroma infiltrated by plasma cells and occasional tissue eosinophil leukocytes. Many of the epithelial cells contained large droplets of mucin. Rather abruptly at one place the mucosa changed into long slender papillae with fibrous stalks covered by large columnar cells with pale vesicular nuclei and acidophilic cytoplasm. From this place the mucous membrane was converted into a large mass of tissue projecting into the lumen; it consisted almost entirely of papillary structures with thin vascular fibrous stalks infiltrated in many places by aggregates of plasma cells, tissue eosinophil leukocytes, and in some places by polynuclear leukocytes. There were only occasional mitotic figures in the lining epithelial cells. Scattered irregularly in the stroma were small aggregates of cross-sections of acini resembling those of Lieberkühn glands. These were lined with tall epithelial cells, a few with large droplets of mucin. In some places in longitudinal and cross-sections of Lieberkühn glands there was a gradual transition from the normal epithelial cell of the mucosa to the larger columnar cell with a paler nucleus which is characteristic of the cells lining the papillary structures. The submucosa contained many mucous glands of Brunner and there were a few small aggregates of these in the tumor tissue. In some places the papillary masses of cells and a few large irregular acini of tumor cells extensively infiltrated the

muscle layer as well as the fibro-areolar tissue and adjacent pancreatic tissue. These tissues also were greatly infiltrated by lymphocytes. The tumor cells in the tissues such as the lymph glands surrounding the duodenum tended to occur in acini rather than as papillary structures.

In the periaortic, peripancreatic and right suprarenal lymph glands there were large nodules of tumor tissue with the same papillary structure as that of the growth in the duodenum. There were also acini formed by tumor cells; in the lumen of many of these were necrotic cells. The normal architecture of all of these glands was destroyed and there was only a small amount of unchanged lymphoid tissue.

The tumor mass in the receptaculum chyli, and the nodules of tumor tissue in the lungs and liver all had the same papillary arrangement as that found in the duodenal tumor.

In diagnosing this tumor as a primary carcinoma of the duodenum, tumors arising from the epithelium of the ampulla of Vater, the common bile duct, the pancreatic duct and the head of the pancreas were considered. As the tumor did not involve the pancreatic tissue extensively and the microscopic structure was unlike the customary tubular or alveolar arrangement of pancreatic carcinomas, this source could be excluded. Its diagnosis as a tumor of the ampulla of Vater or the adjacent common bile duct was opposed by the following features. The lining of the ampulla and common bile duct was everywhere smooth and covered with mucous membrane; the ampulla of Vater was on the edge of the tumor and not near the center as would be expected if it were the primary source. Carcinomas of the ampulla are usually small and confined to that region. These same statements apply in eliminating the pancreatic duct as a source.

PERFORATE POSTERIOR TRICUSPID LEAFLET (Double Tricuspid Orifice). J. J. LUTZ.

Double auriculoventricular orifice is rather a rare condition, and double tricuspid orifice is extremely rare. A second valvular opening, however, supplied with its own cusps, chordae tendinae and papillary muscles may exist within the segments of an otherwise normal auriculoventricular valve. Maude Abbott has a collection of seven specimens, six of which are double mitral orifices, and only one double tricuspid (Osler: *Modern Medicine,* vol. 4, p. 759; Greenfield: *Tr. Path. Soc. London* **27**:128, 1876; Cohn: Inaug. Diss., Konigsburg, 1896; Degen: Inaug. Diss., Greifswald, 1903; Stuhlenweisenburg: *Centralbl. f. allg. Path, u. path. Anat.* **23**:342, 1912; Camisa: *Centralbl. f. allg. Path. u. path. Anat.* **23**: 1027, 1912; Pisenti: Di una rarissma anomalia della tricuspidalis, Paraguae, 1888).

In addition to these seven, there is a specimen of double mitral orifice in the Harvard museum and one in the McGill museum; and another double tricuspid orifice reported by Gutzeit (*Arch. f. path. Anat. u. allg. Path.* **241**:355, 1923).

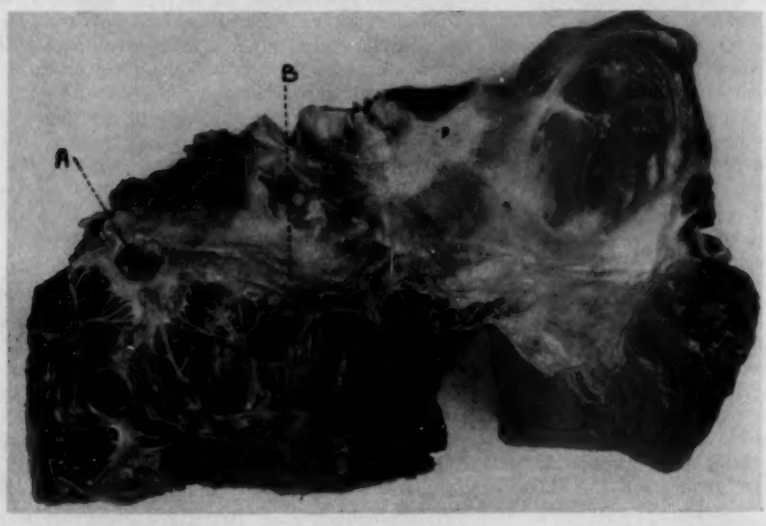
In Stuhlenweisenburg's specimen, and one of Camisa's, the two openings are of equal size and are separated by a bridge of valve tissue which furnishes a cusp to each of the orifices. In the others, the second opening is much smaller than the other and is contained within one of the leaflets. The specimen herein reported is of this type.

The double foramen usually causes no symptoms as it is a functioning valve. In four of the seven specimens reviewed by Abbott, the valves were thin, smooth and competent, while chronic endocarditis was present in the other three.

An unusual traumatic foramen in the anterior leaflet of the tricuspid valve is reported by Lauche (*Arch. f. path. Anat. u. allg. Path.* **241**:16, 1923). The patient, a man, aged 24, came to autopsy with a clinical diagnosis of cardiac failure with edema. On opening the right side of the heart, an oval foramen 0.8 cm. in diameter was found in the anterior leaflet of the tricuspid valve. The edge of the foramen was uniformly thickened, yellowish white and somewhat retracted. Chordae tendinae of normal appearance ran from the lower edge of the opening to the papillary muscles. From gross observations an anatomic diag-

nosis of congenital foramen in the tricuspid valve was made, but microscopic examination revealed evidence of old inflammation and the presence of scar tissue. A careful inquiry into the past history of the patient disclosed that fifteen months previously he had received a stab wound in the second or third intercostal space. After apparent recovery he gradually developed symptoms of cardiac failure and died after a few weeks in the hospital.

The specimen described in this report is similar in size and shape to that reported by Lauche. A white man, aged 43, was first admitted to the hospital on Oct. 13, 1926, complaining of attacks of dizziness, increasing weakness and jaundice of two months' duration. He was perfectly well previous to the onset of this illness. On examination, there was a generalized icteric tinge to the skin, and blood counts showed a severe anemia. There were 2,340,000 red cells, 11,600 white cells and the hemoglobin was 29 per cent. There was bile in the urine and blood in the stools. A transfusion of 400 cc. of blood was given. Although the patient continued to bleed from the bowel, the blood constituents rose. On Dec. 4, 1926, an exploratory operation was performed. A large distended gallbladder



Tricuspid ring laid open (natural size): *A*, opening in posterior leaflet; note chordae tendinae and tendency toward formation of leaflets; *B*, small foramen in septal leaflet.

with multiple adhesions was removed. The patient improved rapidly after the operation, and left the hospital on Jan. 21, 1927. The red cell count was then 4,430,000, the hemoglobin 49 per cent and the white cell count 11,950. There were no symptoms until December, 1927, when weakness and loss of weight reappeared. The patient reentered the hospital on Feb. 12, 1928, with a pronounced secondary anemia, but no changes suggestive of pernicious anemia. Two blood transfusions were given, but the patient responded poorly. He left the hospital in April, very anemic, and returned a month later, on a stretcher, in a semicomatose state. He was again given a transfusion, but died shortly after the transfusion was completed. There were thus no symptoms to indicate any lesions of the valves of the heart.

The heart, with 2 cm. of aorta attached, weighed 345 Gm. on May 21, 1928. It was yellowish red, firm and greasy. The epicardium was yellowish pink, smooth and translucent. There was a layer of fat over the entire heart which was 8 mm. thick over the right auricle, 3 mm. over the right ventricle, 1 mm. over the left

ventricle and 7 mm. at the apex. The coronary arteries were buried in 4 mm. of fat, and were straight and patent throughout. The lining was yellowish pink, studded with yellow, raised patches measuring 1 by 2 mm. These were most numerous in the proximal portions of the vessel. The apex was made up entirely of the left ventricle.

The lining of the right auricle was yellowish pink, smooth and glistening. The foramen ovale was closed. The opening of the coronary sinus was half covered by a thin, semilunar thebesian valve. The wall of the auricle was 2 mm. thick at the base of the heart. The tricuspid ring, opened, measured 14 cm. The leaflets were thin, smooth and translucent. There was a foramen 10 mm. in diameter in the posterior leaflet. A group of chordae tendinae extended from its edges to a small papillary muscle, forming the outline of a cone. In the septal leaflet there was an opening 2 mm. in diameter. The lining of the right ventricle was yellowish pink, smooth and glistening. Shining through it, the myocardium was pale yellowish red, with lighter, more opaque yellow stripes. These changes were seen better in the left ventricle, but occurred most markedly in the papillary muscles of both ventricles. The surfaces made by cutting were pale yellowish red, and the opaque yellow streaks were best seen near the endocardial surface. The lining of the left auricle was similar to that of the right. The wall was 1.5 mm. thick at the base of the heart. The mitral ring, laid open, was 11 cm.; part of it has been cut away. The leaflets were yellowish pink, twice the normal thickness and roughened by fibrous tissue, covered with smooth endothelium, and by slightly raised, opaque yellow patches, some of which were 3 by 2 mm. in diameter. The ventricular cavity measured 10 cm. from apex to pulmonic ring. The pulmonic ring, opened, measured 10 cm. The leaflets were thin, smooth and translucent. As the heart had been opened previously the water test could not be applied. The lining of the left ventricle was similar to that of the right. The streaked appearance was more marked than in the right ventricle. The outer wall was 8 mm. thick at the apex, 15 mm. midway between apex and base of heart, and 20 mm. at the base. The septal wall was 15 mm. thick at the apex, 10 mm. midway between apex and base, and 8 mm. at the base. The ventricular cavity from apex to aortic ring measured 11 cm. The aortic ring, laid open, was 7.5 cm. Part of it had been cut away. The leaflets were thin, smooth and translucent. The lining of the aorta was yellowish pink, studded with slightly raised opaque yellow patches, some of which measured 4 by 5 cm.

In the section of papillary muscle stained with sudan III, 90 per cent of the section contained red-staining globules arranged in transverse and longitudinal bands. In the section through the outer wall of the left ventricle the same amount of red globules was present, but the arrangement in bands is not so marked. In the section of aorta, similarly stained, about 35 per cent of the intima contained red-staining globules.

The anatomic diagnosis was: marked fatty changes of the myocardium, mitral leaflets, and lining of the aorta and coronary vessels; congenital foramina in the posterior and septal leaflets of the tricuspid valve, and slight hypertrophy of the left ventricle.

DISCUSSION

H. G. WELLS: A report of double mitral orifice was made to this Society several years ago. This anomaly was observed also in a dog at about the same time.

E. R. LECOUNT: The hole in the valve described was near the tricuspid ring and not at the margin as were the so-called fenestrations.

Book Reviews

CHRONIC (NONTUBERCULOUS) ARTHRITIS. PATHOLOGY AND PRINCIPLES OF MODERN TREATMENT. By A. G. TIMBRELL FISHER. Price, $8.75. Pp. 232, with 186 illustrations contained in 93 plates (1 colored). London: H. K. Lewis & Company, 1929.

Monographs and papers on arthritis are appearing in ever greater frequency and are often of considerable importance. Fisher, an eminent British surgeon, has approached the problem in a thoroughly logical manner. His discussion of nomenclature leaves much to be desired, but when the classifications proposed are based on mixtures of pathology, etiology and clinical signs it seems impossible as yet to reach any satisfactory conclusions. The chapters on the physiology and pathology of the joints are admirably presented and especially well illustrated. The author bewails the fact that so little is known about the normal physiology of joints and suggests several lines of endeavor in this field. Using the most common English classification of rheumatoid arthritis and osteo-arthritis, Fisher describes the dominant pathologic manifestations of each, at the same time admitting that they may both come to the same end, though by different processes.

The etiology is somewhat briefly but well handled, although the rôle of traumatism in the causation of osteo-arthritis is perhaps overemphasized. When it comes to symptomatology the author makes an attempt to differentiate between the rheumatoid and osteo-arthritic types as clearly as he has pathologically. This differentiation is sometimes not difficult, but more often, at least in the advanced stages, the clinical picture may be so confusing as to make this impossible. Finally in the chapter on treatment Fisher discusses several lines of attack: medical, dietary and surgical. There is considerable space devoted to surgical treatment, and this portion bears the imprint of a capable and experienced surgeon.

On the whole this book is a splendid contribution to the subject, and is particularly stimulating in promoting further observations on the etiology, pathologic changes and normal physiology in their relationship to chronic articular processes.

MANSON'S TROPICAL DISEASES: A MANUAL OF THE DISEASES OF WARM CLIMATES. Edited by PHILIP H. MANSON-BAHR, M.D., Physician to the Hospital for Tropical Diseases, London. Ed. 9. Revised. Price, $11. Pp. 921, with 35 plates, 401 figures in the text, 6 maps, and 34 charts. New York: William Wood & Company, 1929.

The purpose of Patrick Manson in writing his book was to present in handy form essential information about the diseases of warm climates. The appearance at this time of the ninth edition, revised, shows that the book has proved to be of large practical service. The editor of this edition has done his part well. Much new knowledge has been incorporated and, when necessary, as in the case of yellow fever, previously accepted views and ideas have been abandoned. The general arrangement of the text is unaltered and the number of pages is about the same as in preceding editions. To quote from the preface: "Attention has been especially devoted to the subject of treatment—now, happily, becoming more and more stabilized—in recognition of the paramount position that clinical study and clinical methods still hold in Tropical Medicine."

A special section is devoted to the technic of injections, including transfusion of blood, in the treatment of tropical diseases. Medical zoology and laboratory methods are considered in the appendix which comprises more than 200 pages. The illustrations, almost without exception, are instructive and useful. The book will continue to be of practical service not only to medical students and physicians in tropical places, but to all who may be interested in the diseases with which it deals.

LEHRBUCH DER TOXIKOLOGIE FÜR STUDIUM UND PRAXIS. VON FERDINAND FLURY, Professor der Pharmakologie an der Universität Würzburg, und HEINRICH ZANGGER, Professor der gerichtl Medizin an der Universität Zurich. Paper. Price, 29 marks. Pp. 500, with 9 illustrations. Berlin: Julius Springer, 1928.

This work is primarily intended as an introduction to the study of toxicology for students and as a short reference work for practitioners, although it should prove of interest to special workers in this field. The book is divided into two parts: the general part, in which a rather extensive discussion is given of the general principles of toxicology, statistics of poisoning and certain legal requirements in connection with poisons; and the special part, in which are handled the specific groups of poisons. The discussions in the general part are, for the most part, adequately presented, although the section dealing with the methods of detection would seem to be somewhat curtailed, especially when little or no attention is given to this field under the specific poisons. It is possible, however, that this phase of the subject may be properly shortened in a work of this character, intended for students who will, perhaps, never have occasion to make any tests for the poisons in question. The special part is well arranged and presented, the discussions being clear and concise, although special emphasis is given to symptomatology and treatment. Especially commendable in this part is the section by Zangger dealing with the gaseous poisons. The subject matter in this special part is presented and arranged in such a manner that the student should have little difficulty in correlating and assimilating it. This book should prove a valuable addition to the library of those who wish a short work on the subject.

CONSTITUTIONAL INADEQUACIES. By NICOLA PENDE, M.D. Translated by SANTE NACCARATI, M.D., Sc.D., PH.D., with a foreword by GEORGE DRAPER, M.D. Price, $3.50. Pp. 270. Philadelphia: Lea & Febiger, 1928.

A review of our present knowledge in constitutional pathology is presented in this small volume. The investigations and conceptions of the Italian School on this subject are especially stressed. After a definition of the term constitution, its three aspects (morphologic, dynamic-humoral and psychologic) are discussed. Two main constitutional types (megalosplanchnic-hypervegetative and microsplanchnic-hypovegetative) are recognized, from which numerous variants and mixtures exist. In the second part of the book the author discusses the localized constitutional anomalies and inadequacies according to the organic systems (skin with appendages, skeletomuscular system, blood and hemolymphopoietic system, circulatory apparatus, respiratory apparatus, digestive apparatus, urogenital apparatus, nervous system and the endocrine system). A chapter dealing with the therapy of constitutional inadequacies concludes the presentation. The book illustrates very well the importance of the individual constitution in health and disease. While the conclusions reached by the author are not always convincing as they are lacking proper support by sufficient and well recognized observations and facts, the book is highly stimulating. There is no bibliography.

LECTURES ON PLANT PATHOLOGY AND PHYSIOLOGY IN RELATION TO MAN. A series of lectures given at the Mayo Foundation and the Universities of Wisconsin, Minnesota and Iowa, the Des Moines Academy of Medicine, and Iowa State College in 1926 and 1927. Cloth. Price, $2.50. Pp. 207, with illustrations. Philadelphia: W. B. Saunders Company, 1928.

The subjects discussed are: filtrable viruses, by L. O. Kunkel; ecology and human affairs, by H. C. Cowles; some aspects of the problem of the fusarium, by G. H. Coones; racial specialization in the fungi of plant disease, by E. C. Stakman; the relation of plant pathology to human affairs, by H. H. Whetzel; and some aspects of cellular physiology, by W. J. V. Osterhout. The lecturers are successful investigators in their respective fields, and their presentations will interest physicians and others who are concerned in the advances of biologic science.

Books Received

SURGICAL PATHOLOGY. By CECIL P. G. WAKELEY, F.R.C.S. (Eng.), F.R.S. (Edin.), and ST. J. D. BUXTON, M.B., B.S. (Lond.), F.R.C.S. (Eng.). Price, $12.50. Pages 904, with 392 illustrations. New York: William Wood & Company, 1929.

PATHOLOGY FOR STUDENTS AND PRACTITIONERS. Authorized Translation of the "Lehrbuch der pathologischen Anatomie." By DR. EDWARD KAUFMANN, Professor of General Pathology and Pathological Anatomy, University of Göttingen. Translated by Stanley P. Reimann, M.D., Pathologist and Director of the Research Institute of the Lankenau Hospital. In three volumes. Cloth. Price, $30 per set. Pp. 2,452, with 1,072 illustrations. Philadelphia: P. Blakiston's Son & Company, 1929.

REPORT OF THE MEDICAL RESEARCH COUNCIL FOR THE YEAR 1927-1928. Price, 3 shillings. Pp. 165. London: His Majesty's Stationery Office, 1929.

LABORATORY TECHNIQUE. The Methods Employed at St. Luke's Hospital, New York. By F. C. Wood, Karl Vogel and L. W. Famulener. Third edition, revised and enlarged. Price, $3.75. Pp. 318. New York: James T. Dougherty, 1929.

OLD AGE. The Major Involution. The Physiology and Pathology of the Aging Process. By ALDRED SCOTT WARTHIN, Ph.D., M.D., LL.D., Professor of Pathology and Director of the Pathological Laboratories in the University of Michigan, Ann Arbor. Cloth. Price, $3, net. Pp. 200, with 29 illustrations. New York: Paul B. Hoeber, 1929.

ETIOLOGIE ET PROPHYLAXIE DE LA GRIPPE. Bacille de Pfeiffer virus filtrant grippal. Par P. DUJARRIC DE LA RIVIÈRE. (Monographies de l'Institut Pasteur.) Price, 32 francs. Pp. 108, with 21 illustrations. Paris: Masson & Cie, 1929.

A MANUAL OF HELMINTHOLOGY, MEDICAL AND VETERINARY. By H. W. BAYLIS, M.A., D.Sc., Assistant Keeper, Department of Zoology, British Museum (Natural History). Price, $10. Pp. 303, with 200 illustrations. New York: William Wood & Company, 1929.

PROTOZOOLOGY, A MANUAL FOR MEDICAL MEN. By JOHN GORDON THOMSON, M.A., M.B., Ch.B., Director, Department of Protozoology, London School of Hygiene and Tropical Medicine, and Andrew Robertson, M.B., Ch.B., Lecturer and Milner Research Fellow in the Department of Protozoology, London School of Hygiene and Tropical Medicine. Price, $11. Pp. 376, with 224 illustrations. New York: William Wood & Company, 1929.

ARTHRITIS AND RHEUMATOID CONDITIONS: Their Nature and Treatment. By RALPH PEMBERTON, M.S., M.D., Physician to the Presbyterian Hospital, Philadelphia, and Associate Professor of Medicine in the Graduate Medical School of the University of Pennsylvania. Price, $5, net. Pp. 354, with 43 illustrations. Philadelphia: Lea & Febiger, 1929.

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